# GENETICS

*A periodical record of investigations bearing on heredity and variation*

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## Effective with Volume 45, 1960

1. GENETICS will be changed from a bimonthly to a monthly journal.

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# THE NATURE OF ASYMMETRY AND VARIABILITY IN THE DOUBLE BAR-EYELESS$^2$ DROSOPHILA

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Received November 14, 1958

IT is a common observation that the corresponding parts of bilateral symmetrical animals are never absolutely the same size. Many of the genetic traits which thus far have been studied have indicated a varying degree of asymmetry of the parts affected. Many genetically controlled morphological traits in animals as well as in humans oftentimes show unilateral expression. This is commonly observed in polydactylism and brachydactylism.

This asymmetrical condition poses an interesting problem. Presumably, all the cells in an animal body have the same genetic constitution, barring the less common events such as somatic mutations, somatic crossing over and other rare chromosomal events which can occur during development and which can bring about phenotypic differences. If this basic hypothesis is correct, then the corresponding parts of an animal have cells with the same genetic constitution. The difference in the morphology of the corresponding parts then must lie in the differences of environmental factors, both internal and external.

At first, the problem to rigidly control these factors on one side of the animal and vary them on the other, from egg to adult stage, seems hopeless. The following brief paper describes an experiment in which this problem may be approached more easily and perhaps shed some light on the problem of asymmetry, variability, unilateral expression, and the different degrees of expressivity of genetic traits.

## *The method of investigation*

A small organism like the fruit fly lends itself especially well to this study. The mode of attacking this problem has been to study the variation of eye sizes in the BB-eyeless stock. Female flies heterozygous for double Bar and homozygous for eyeless (*BB*/+, $ey^2/ey^2$) were synthesized. The trait eyeless is selected because of its large variation of eye sizes, ranging from no eye to a complete full eye, and BB factor is added to decrease the size of the eye in order to facilitate the task of counting the facets in each eye. The number of facets in each eye is used as an index for determining the size of the eye. In the early part of the experiment an attempt was made to obtain females homozygous for double Bar and eyeless (*BB*/*BB*, $ey^2/ey^2$) for the purpose of this study. However, a large number of these flies have eyes with pigmented areas but completely devoid of facets. Moreover, these females are sterile, and therefore, unable to form a homozygous stock. A similar condition occurs in the *BB*, $ey^2/ey^2$ males. All the study on asym-

metry, therefore, has been carried out on *BB/+,ey²/ey²* females, since these proved most favorable from the standpoint of eye size and fertility.

### *The data*

*Data at 25°C:* A total of 2,650 flies was raised at 25°C and their eye sizes determined. Table 1 shows the numerical distribution of eye size as found on the left and right sides. Statistical tests have shown that the distributions on the two sides are not significantly different from one another. Figure 1 shows the plotted data. Only the total plotting is shown here. The $x$ axis represents the size of the eye in terms of facet number and the $y$ axis, on a logarithmic scale, the number of eyes in each group. The points seem to conform to a negative exponential relationship of $y=ab^{-x}$, where $a$ and $b$ are constants. There seems to be a definite break in the line between the points for the 12th and 13th faceted eye size. An understanding of this break in the distribution is not clear at the present.

When each of these points is further analyzed, still different classes of distributions are revealed. Table 2 shows the numerical distributions of eye sizes on one side of the head when the other side is kept at a fixed size. The horizontal rows, L0, L1, L2—etc., refer to each group in which the left eye had zero facets, one facet, two facets—etc. The vertical columns, R0, R1, R2—etc., refer to groups of right eyes with 0, 1, 2—facets. For example, taking the group L0 with zero facets in the left eye, it is found that 797 eyes have zero facets on the right, 101 eyes have one facet on the right, 67 eyes have two facets—etc. Some of these have been plotted and the graph is shown as Figure 2. Here one can observe a relatively uniform transition from one distribution to another, ranging from a highly

TABLE 1

*Numerical distribution of eye size on the left and right side of* BB/+, ey²/ey² *females at 25°C*

| No. of facets in each eye | No. of left eyes | No. of right eyes | Total left and right |
|---|---|---|---|
| 0 | 1113 | 1172 | 2285 |
| 1 | 267 | 228 | 495 |
| 2 | 217 | 232 | 449 |
| 3 | 192 | 174 | 366 |
| 4 | 128 | 152 | 280 |
| 5 | 143 | 155 | 298 |
| 6 | 98 | 104 | 202 |
| 7 | 103 | 81 | 184 |
| 8 | 80 | 71 | 151 |
| 9 | 71 | 55 | 125 |
| 10 | 48 | 45 | 93 |
| 11 | 40 | 50 | 90 |
| 12 | 40 | 26 | 66 |
| 13 | 16 | 13 | 29 |
| 14 | 9 | 12 | 21 |
| 15 | 17 | 12 | 29 |
| 16 | 9 | 9 | 18 |

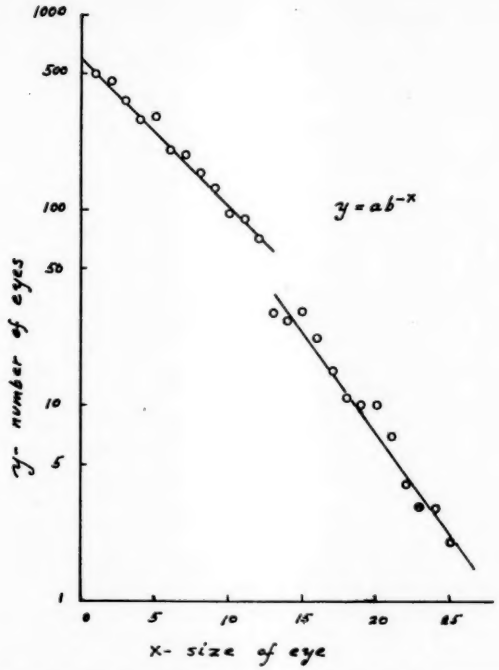


FIGURE 1.—Graph showing distribution of eye sizes of double Bar eyeless (*BB/+, ey²/ey²*) females at 25°C.

TABLE 2

*Distribution of symmetrical and asymmetrical eye sizes in* BB/+, ey²/ey² *female flies at 25°C*

| | | Right eye | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | R0 | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | R11 |
| Left eye | L0 | 797 | 101 | 67 | 38 | 35 | 29 | 16 | 12 | 9 | 5 | 2 | 2 |
| | L1 | 139 | 32 | 29 | 16 | 12 | 14 | 8 | 7 | 3 | 1 | 1 | 3 |
| | L2 | 66 | 36 | 47 | 27 | 15 | 9 | 4 | 3 | 0 | 3 | 1 | 2 |
| | L3 | 57 | 15 | 23 | 22 | 26 | 22 | 10 | 4 | 3 | 4 | 3 | 2 |
| | L4 | 28 | 10 | 20 | 10 | 10 | 13 | 14 | 8 | 5 | 3 | 1 | 3 |
| | L5 | 32 | 10 | 12 | 20 | 12 | 15 | 8 | 10 | 9 | 4 | 3 | 2 |
| | L6 | 12 | 8 | 13 | 8 | 12 | 11 | 7 | 3 | 9 | 6 | 2 | 4 |
| | L7 | 17 | 6 | 7 | 11 | 11 | 12 | 8 | 5 | 6 | 5 | 4 | 7 |
| | L8 | 10 | 4 | 5 | 7 | 4 | 9 | 4 | 6 | 7 | 5 | 5 | 1 |
| | L9 | 5 | 1 | 3 | 7 | 4 | 5 | 9 | 6 | 8 | 9 | 6 | 0 |
| | L10 | 3 | 1 | 3 | 1 | 2 | 6 | 4 | 4 | 4 | 3 | 4 | 7 |
| | L11 | 0 | 1 | 1 | 4 | 3 | 1 | 5 | 5 | 4 | 1 | 4 | 6 |

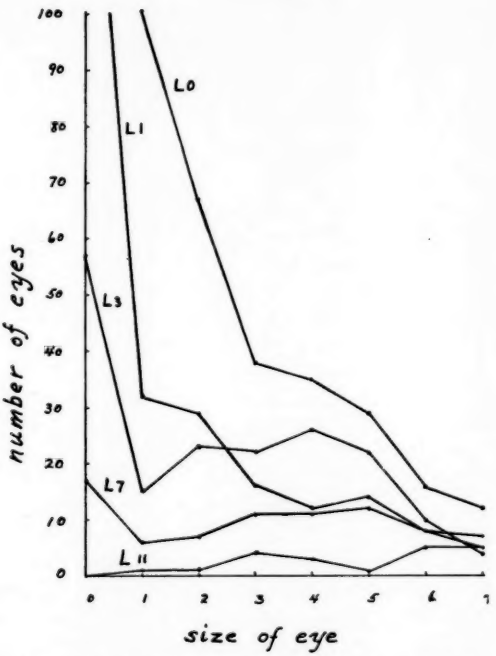


FIGURE 2.—Graph showing distributions of eye sizes on the right side in *BB*/+, $ey^2/ey^2$ females when the left is kept at a fixed size, L0, L1, L3, L7, L11. Skewness decreases with increasing size of the left eye. (See Table 2).

skewed curve to a modal or perhaps a multimodal type of curve. A detailed mathematical study of these curves might prove interesting but not particularly pertinent to the point in question. These curves simply point out the natural range and distribution of eye sizes within each group. The tail ends of these curves are not shown; however, the largest right eye found, for example, in group L0, had 11 facets, while the largest eye found in group L11 had 16 facets (this last value is not shown in Table 2). Simple percentage calculations made from the data presented in Table 2 reveal that, among all flies showing facets in either one or both eyes, approximately nine percent show symmetry, 91 percent show asymmetry. This overwhelming number of asymmetrical flies cannot be attributed to mere accidental causes in the environment, but must have a more deep-seated rational explanation. The work of CHEN (1929), MEDVEDEV (1935), BODENSTEIN (1942), STEINBERG (1943) and DEMARINIS (1943) have all indicated that the rudiment of the eye has its beginning during the egg stage. On the basis of this knowledge, the cause of these distributions is assumed to occur very early in development—perhaps as early as the time of the migration of the cleavage nuclei (about the first two hours of the egg development, based on the best available

data presented by SONNENBLICK (1950). The eyeless gene may act during this period to change the distribution of the normal random migration of these nuclei to the presumptive left and right eye regions of the oöplasm. The distribution of these cleavage nuclei are reflected in the distribution of the adult eye sizes. Figure 3 shows a schematic drawing of this interpretation.

*Data at 17° and 28°C:* This tentative migration hypothesis was tested by running two more series of flies at two different temperatures. In one series, flies were allowed to deposit eggs for a period of ten hours at 17°C and then these eggs were transferred to 25°C for the remainder of their developmental period. In another series, the eggs were allowed to develop at 28°C for the first ten hours and then transferred to 25°C for the rest of the developmental period. In each case, the eggs deposited during the last hour of the egg-laying period did not receive the complete test temperature. The first ten hour period of development was chosen since this period amply covers the time of migration of the cleavage nuclei as described by SONNENBLICK (1950), and yet does not overlap with the TEP of either BB factor, as shown by DRIVER (1931), or the $ey^2$ gene, as shown by BARON (1935). Any change in the distribution of these cleavage nuclei by a change in temperature would be reflected in the distribution of the adult eye size. Tables 3 and 4 list the distributions of eye size obtained at these two temperatures. Figure 4 illustrates the plotted data obtained in these two series and compares them with the data previously obtained at 25°C. Table 5 lists the calculated values of the two constants for each curve. Calculation shows a significant difference at one percent level between the two regression coefficients log b=0.07738 at 25°C and log b=0.16598 at 17°C, and also between the two regression coefficients log b=0.07738 at 25° and log 0.15236 at 28°, indicating a significant shift in the distribution of eye size, hence, seemingly supporting the migration hypothesis. However, no significant difference is observed between the 17°C and the 28°C series. A significant shift may be noted by calculating the ratio between faceted and nonfaceted eyes for each temperature series as shown in Table 6. The ratio

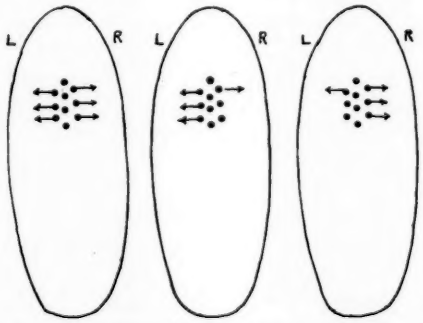


FIGURE 3.—Schematic drawing showing possible asymmetrical migration of cleavage nuclei in $BB/+$, $ey^2/ey^2$ females. The $ey^2$ gene acts at this time to interfere with the normal migration distribution of these nuclei to their presumptive eye region.

TABLE 3

*Numerical distribution of eye size on the left and right side of* BB/+, $ey^2/ey^2$ *females raised at 17°C during the first ten hours of development and completed at 25°C*

| No. of facets in each eye | No. of left eyes | No. of right eyes | Total left and right |
|---|---|---|---|
| 0 | 426 | 455 | 881 |
| 1 | 95 | 72 | 167 |
| 2 | 73 | 68 | 141 |
| 3 | 43 | 37 | 80 |
| 4 | 28 | 27 | 55 |
| 5 | 16 | 22 | 38 |
| 6 | 11 | 12 | 23 |
| 7 | 13 | 14 | 27 |
| 8 | 9 | 6 | 15 |
| 9 | 5 | 6 | 11 |
| 10 | 6 | 2 | 8 |
| 11 | 0 | 2 | 2 |
| 12 | 2 | 1 | 3 |

TABLE 4

*Numerical distribution of eye size on the left and right side of* BB/+, $ey^2/ey^2$ *females raised at 28°C during the first ten hours of development and completed at 25°C*

| No. of facets in each eye | No. of left eyes | No. of right eyes | Total left and right |
|---|---|---|---|
| 0 | 196 | 192 | 388 |
| 1 | 28 | 37 | 65 |
| 2 | 31 | 19 | 50 |
| 3 | 21 | 17 | 38 |
| 4 | 10 | 14 | 24 |
| 5 | 6 | 14 | 20 |
| 6 | 5 | 7 | 12 |
| 7 | 4 | 5 | 9 |
| 8 | 7 | 3 | 10 |
| 9 | 5 | 6 | 11 |
| 10 | 1 | 0 | 1 |

TABLE 5

*Calculated values of constants* a *and* b *for each curve,* $\log y = \log a - x \log b$

| | Constant, $\log a$ | Constant, $\log b$ |
|---|---|---|
| Upper part, curve (1) 25°C | 2.79692 | 0.07738 |
| Lower part, curve (2) 25°C | 2.93049 | 0.10303 |
| Curve (3) at 17°C | 2.43611 | 0.16598 |
| Curve (4) at 28°C | 2.03635 | 0.15236 |

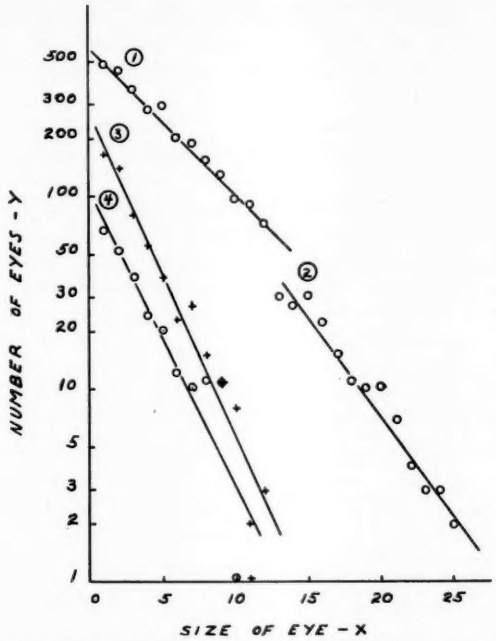


FIGURE 4.—Graph showing distribution of eye sizes in the *BB*/+, $ey^2/ey^2$ females. Curves 1 and 2 represent complete development at 25°C. Curve 3, first ten hours of development at 17°C and completed at 25°C. Curve 4, first ten hours of development at 28°C and completed at 25°C.

TABLE 6

*Ratios between faceted and nonfaceted eyes in* BB/+, $ey^2/ey^2$ *female flies raised at three different temperatures during the first ten hours of development*

| | Number of faceted eyes | Number of nonfaceted eyes | Ratio of faceted to nonfaceted eyes | Total number of flies |
|---|---|---|---|---|
| Complete period of development at 25°C | 3013 | 2287 | 1.317 | 2650 |
| First 10 hours of development at 17°C, completed at 25°C | 581 | 881 | 0.650 | 731 |
| First 10 hours of development at 28°C, completed at 25°C | 253 | 389 | 0.650 | 321 |
| | | | | 3702 |

is significantly higher for the 25°C series than that for either 17° or 28°C series. (Deviation between 17° and 25°C, $\chi^2 = 159$, $P<0.01$ and between 28° and 25°C, $\chi^2 = 70.6$, $P<0.01$). At the time the experiment was set up the greatest shift was expected between 17° and 28°C. However, the present data show no difference

between the two temperatures. While it is true that protoplasmic viscosity is often affected by a change in temperature, it must be realized that such relations are often complex in protoplasm, and obtaining results such as the above is not at all surprising.

Tables 7 and 8 show the numerical distributions of eye sizes on one side of the head when the other side is kept constant. These may be compared with the 25°C series reported in Table 2.

*Other pertinent data:* In an earlier series, the eggs were allowed to develop at 30°C during the first ten hours of development and then transferred to 25°C for the rest of their developmental period. It was found that flies kept at this temperature (30°C) became sterile within 24 hours, and when transferred to 22°C they

TABLE 7

*Distribution of eye sizes, in terms of facet number in* BB/+, $ey^2/ey^2$ *females raised at 17°C during the first ten hours of development and completed at 25°C*

| | | Right eye | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | R0 | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 |
| Left eye | L0 | 315 | 39 | 28 | 13 | 6 | 9 | 5 | 7 | 0 | 2 | 0 |
| | L1 | 51 | 13 | 11 | 8 | 5 | 2 | 1 | 1 | 2 | 1 | 0 |
| | L2 | 36 | 13 | 11 | 5 | 3 | 2 | 0 | 1 | 2 | 0 | 0 |
| | L3 | 20 | 1 | 8 | 5 | 3 | 2 | 1 | 0 | 0 | 1 | 0 |
| | L4 | 14 | 2 | 1 | 2 | 4 | 2 | 0 | 2 | 1 | 0 | 0 |
| | L5 | 5 | 1 | 0 | 2 | 3 | 2 | 1 | 0 | 0 | 0 | 1 |
| | L6 | 2 | 1 | 4 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 |
| | L7 | 3 | 0 | 2 | 1 | 2 | 0 | 4 | 1 | 0 | 0 | 0 |
| | L8 | 5 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| | L9 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| | L10 | 2 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 |

TABLE 8

*Distribution of eye sizes, in terms of facet number, in* BB/+, $ey^2/ey^2$ *females raised at 28°C during the first ten hours of development and completed at 25°C*

| | | Right eye | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | R0 | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 |
| Left eye | L0 | 144 | 21 | 8 | 8 | 4 | 6 | 2 | 1 | 1 | 1 | 0 |
| | L1 | 17 | 5 | 1 | 3 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| | L2 | 11 | 3 | 5 | 3 | 4 | 2 | 1 | 1 | 0 | 1 | 0 |
| | L3 | 10 | 2 | 1 | 2 | 2 | 2 | 1 | 1 | 0 | 0 | 0 |
| | L4 | 3 | 1 | 0 | 1 | 1 | 1 | 0 | 2 | 1 | 0 | 0 |
| | L5 | 2 | 1 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | L6 | 2 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| | L7 | 0 | 1 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 |
| | L8 | 2 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 0 |
| | L9 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 |
| | L10 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

did not regain their fertility. These were discarded and another series started at 28°C. These, too, became completely sterile by the end of 72 hours. However, enough $F_1$'s were obtained from this series to test the validity of the proposed hypothesis.

A large number of eyes recorded as having no facets at all, as shown in Tables 1, 3 and 4, has in most cases, a pigmented area (red) of approximately the same size and outline as those having facets. In those where the eyeless factor was expressed more fully, the shape of the heterozygous *BB* was reduced considerably. There seems to be no obvious correlation between the size of the pigmented area and the number of facets. Most often the pigmented area was shiny and smooth, similar to the surface of the head hypodermis. These nonfaceted red areas are not unusual. The revealing histological work of WOLSKY and HUXLEY (1936) on the nonfaceted regions of the bar eye have demonstrated that these areas lack the dioptric apparatus and the retinule cells and have only the pigment-bearing accessory cells. A possible explanation for these nonfaceted areas is given in the discussion. In a relatively few cases the area is limited to the upper lobe of the eye. These too, may or may not show facets. Occasionally an antenna was found growing out of the normal position of the eye.

## DISCUSSION

*Pigmented and nonfaceted eyes:* The data presented here seem to indicate that the asymmetry of eye size is best interpreted on the basis of a change in the random distribution of the cleavage nuclei during the migration period of the egg stage. This interpretation may be further extended to explain also the large number of eyes with pigmented and nonfaceted tissue (referred in the data as eyes with zero facets). In this case, probably the presumptive eye oöplasm, devoid of nuclei, is filled in by other nuclei derived from the subsequent nuclear divisions of the adjacent nuclei during the blastema stage. This condition, undoubtedly, introduces a different biochemical chain reaction in development which may finally lead to pigmented and nonfaceted tissue in the adult stage. One would need more positive data, however, to confirm this interpretation.

*Chance variation on a localized level:* The data show that approximately 91 percent flies are asymmetrical and nine percent symmetrical. The overwhelming number of asymmetrical flies cannot be easily explained by the less common genetic events such as somatic crossing over, mutation and other chromosomal aberrations. Further, it is reasoned that the external environment is fairly uniform on the left and right side of any one single organism. This too may be eliminated as being the chief cause of asymmetry. A comparison of eye sizes found in the eyeless stock will immediately convince one of this. The cause of asymmetry, therefore, must lie in the difference in interaction between the genetic constitution and the cytoplasm on a very localized level.

Up to this point, it is surmised that asymmetry is neither caused by a difference in the genetic constitution nor by difference in the external environment, implying, of course, that two identical genetic conditions reacting under two identical

sets of environmental conditions do not necessarily lead to two identical phenotypes (referring to the corresponding left and right half of an individual). It seems that chance still plays its role in producing variation. This problem has been studied by other workers. MATHER (1953) analyzing a similar problem by using the number of sternopleural chaetae in Drosophila as the index of asymmetry, arrived at the same conclusion. He referred to asymmetry "as a reflection of instability" caused by "chance disturbances", "local disturbances", or "local upsets". GOLDSCHMIDT (1955), in his recent excellent book on theoretical genetics, refers very briefly to some of his unpublished data on the action of modifiers on the asymmetry of dominant Beaded mutant. He states "that the asymmetry is not one of the features of the action of the mutant locus but of some independently determined condition of the reaction system." REEVE (1953) came to the same conclusion in his study of the variation of chaetae number of the abdominal sternites in Drosophila. He concluded "that the nongenetic variance of chaetae number is mainly, in our culture conditions, a 'chance' variability of extremely localized origin, and is not a true environment variability".

Chance variation on a very localized level, as the ultimate cause of asymmetry, is best explained in terms of statistical energy levels. Phenotypic differences brought about by differences in these varying energy states between the genetic constitution and the cytoplasm, should be considered in any problem dealing with variation, asymmetry, unilateral expression and the different degrees of expressivity. Further, in evolution, this source of variation may even have a temporary effect in bridging a species from generation to generation until such time as proper mutations occur or permanent changes in the environment take place.

## SUMMARY

Asymmetry of eye sizes has been studied in the double Bar-eyeless (*BB*/+, $ey^2/ey^2$) in *Drosophila melanogaster*. The general distribution of eye size *vs.* number approximated $y=ab^{-x}$, where $y$=number of eyes, $x$=eye size, and $a$ and $b$ are constants. A further analysis of each group shows types of distribution different from the general trend. Each distribution changes with definite regularity from group to group, being highly skewed in the lower range of eye size and approaching a modal type of distribution in the higher range. Temperature changes during the first ten hours of development have indicated a marked shift in these distributions. The distributions of eye size are tentatively interpreted on the basis of the distributions of the cleavage nuclei during their migration period. The eyeless$^2$ gene may act at this time to change the normal random distribution of these nuclei.

## ACKNOWLEDGMENT

I wish to express my thanks to PROF. WALTER R. VAN VOORHIS and PROF. ALLEN W. BRUNSON of the Mathematics Department of Fenn College for their help given in certain mathematical aspects of this work.

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# STUDIES IN QUANTITATIVE INHERITANCE. XIII. INTERRELATIONS BETWEEN GENETIC BEHAVIOR AND DEVELOPMENT IN THE CELLULAR CONSTITUTION OF THE DROSOPHILA WING

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THE preceding paper of this series (ROBERTSON 1959) and the present one deal with different parts of a single study of the relations between cell size and number in the wing of *Drosophila melanogaster*. The reasons for examining this problem together with details of procedure and statistical analysis are given in the earlier paper but a brief resumé will help the reader to appreciate what follows.

Wing size is highly correlated with body size. Since it is possible to estimate cell size and number in the wing, we can follow changes in the cellular constitution of the wing which accompany the alteration of body size for either genetic or environmental reasons. The wing is made up of a double layer of cells, each of which carries a tiny bristle so that the bristle density in a given area on one surface of the wing provides an estimate of cell area. The evidence presented in the earlier paper showed that variation in the pattern of bristle density in different regions of the wing is relatively unimportant in the material studied in these experiments and that the bristle density in a central region of the wing provides an adequate measure of general cell area or cell size. The records of wing and cell area are expressed in natural logarithms of squared hundredths of a millimeter. Cell number can be estimated from the difference between log wing and log cell area.

The effects of environmental variation were studied by growing larvae at different temperatures and also on chemically defined, aseptic media, deficient in some essential nutrient, which led to smaller wing and body size. Lower temperature during growth results in larger body size; the observed differences in wing size are expressed almost entirely by changes in cell size and the average estimated cell number is about the same at high and low temperatures, which are responsible for big differences in wing area. On the other hand, when the larval diet is suboptimal, provided it is not too deficient, there is a distinct tendency for cell size to remain constant and cell number to decline. But with more extreme conditions, cell size declines as well. Thus equivalent changes in total wing and body size, resulting from different kinds of environmental change are associated with different relations between cell size and number.

The relative contribution of genetic effects to the total within-culture variance for various wild populations grown under favorable conditions at 25°C, was derived by comparing variance and covariance components of the wild flies with

corresponding estimates based on genetically uniform crosses between inbred lines. Most of the variance is genetic and it was inferred that genetic variation of wing size is associated primarily with variation in cell number, but there is also an appreciable genetic correlation with cell size as well; the status of this correlation was left for consideration in the present paper.

Interpretation of the relations between cell size and number and variation of wing size is complicated by the fact that genetically different individuals of identical wing and body size, may differ in cell size and number in the wing. Genetic variation in such inverse relations between cell size and number was studied by selecting for large and small cell size in three different wild populations. It was possible to shift the cell size and number relation more easily in favor of larger cells and fewer of them than in the opposite direction. Whereas continued selection for larger cells failed to effect a permanent increase in body size, selection for small cells lead to a decline. Furthermore, in a cross between a small strain selected for small cells and the unselected stock, the genetic differences in body size behaved as entirely recessive or hypostatic. Such behavior contrasts with the more nearly additive behavior found when strains selected for small body size are backcrossed to the unselected population. It was suggested that the selection for small body size, and selection for small cell size involve different kinds of developmental change which is reflected in genetic behavior. It was suggested that selection for small cell size provides a direct way of breaking up epistatic gene combinations which normally ensure the stability of body size in a population.

Evidence of this kind provides a valuable link between the methods and concepts of population genetics and developmental genetics, extends the depth of analysis and brings various dynamic aspects of growth within reach of genetic study.

The present paper continues the analysis and is concerned especially with the changes in cell size and number which are associated with different kinds of genetic change in wing size.

## ANALYSIS OF DATA

### *Selection for body size*

It has been noted that the estimates of total genetic variance and covariance described in the preceding paper suggested that most of the genetic variation of wing size is due to variation of cell number. It is now necessary to test this inference more directly. To produce differences for comparison, mass selection for long and short thorax length was carried out for three generations in three different populations—Pobla de Lillet, Renfrew and Crianlarich, in which two similar selection experiments were carried out at different times.

Thorax rather than wing length was chosen as the measure of body size, to avoid changes which might alter the characteristic wing/thorax ratio and thereby introduce an additional, irrelevant factor. In addition, two large and two small strains, started at the same time from the Pacific population, have been selected

for five generations. It is convenient to consider them separately, so we shall deal first with the lines selected for three generations. The general procedure in such selection experiments, in which 20 pairs of parents are selected each generation from 100 pairs, together with the effects of long term selection, have been described elsewhere (ROBERTSON 1955).

*Strains selected for three generations:* As expected, selection was immediately effective in both directions and, at the time of comparison, the wing size of the selected strains differed appreciably from the control levels (Table 1). There was

TABLE 1

*Effects of three generations of selection for large and small body size*

| Selected strains | Deviation from unselected, in log units | | |
|---|---|---|---|
| | Wing area | Cell area | Cell number |
| Crianlarich (1st test) | | | |
| L | .0580** | —.0115 | .0695 |
| S | —.0732** | .0175* | —.0907 |
| Crianlarich (2nd test) | | | |
| L | .0299** | —.0319** | .0618 |
| S | —.1018** | —.0348** | —.0670 |
| Renfrew | | | |
| L | .0212* | .0016 | .0196 |
| S | —.0518** | —.0018 | —.0500 |
| Pobla de Lillet | | | |
| L | .0309** | .0131 | .0178 |
| S | —.0504** | .0087 | —.0591 |
| Average deviation | | | |
| L | .0350 | —.0072 | .0422 |
| S | —.0693 | —.0026 | —.0667 |

* Indicates the difference significant at .05 level of probability.
** Indicates the difference significant at .01 level of probability.

a distinct tendency for selection downward to be more effective than selection upward—a familiar experience from the earlier selection experiments. Although wing and hence general body size changed with selection, cell size failed to show any consistent tendency to change in the same direction as the wing size. In the first Crianlarich test, cell size was significantly greater in the small strain; in the second test both selected strains had smaller cells while in the Renfrew and Pobla de Lillet series the changes in cell size were trivial. Averaging wing and cell size deviations for the strains selected in the same direction, it appears that a difference of about ten percent in wing area leaves cell size unchanged. Since there is plenty of variation which can alter the cell size and number relations, independently of wing size, limited more or less random changes in these relations are to be expected when any character is selected for. Hence, the response to selection for large and small size, at any rate in the early stages of selection, are associated with changes in cell number. But the analysis presented in the preceding paper indicated an appreciable genetic correlation between wing and cell area and yet

there is no evidence of this in the comparisons between selected strains. It may be inferred, therefore, that this correlation is due to nonadditive effects. Naturally, effective selection depends primarily on additive effects and this suggests that the variation of wing size in a wild population is influenced by qualitatively different kinds of developmental variation with characteristically different genetic properties.

The evidence from these experiments agrees with the observation of ZARAPKIN (1934) who found that selection for large and small wing size in *D. funebris* was accompanied by change in cell number. But it does not agree with the results of two-way selection for wing length, reported in the first number of this series (ROBERTSON and REEVE 1952), which resulted in correlated changes in cell size. However, selection for small wing size lead to a disproportionate reduction of that organ, compared with thorax length, due especially to sex-linked effects. It is fairly certain now that the observed differences in cell size were associated with this disproportionate change of wing size, whereas, in the present experiments, selection has been for changes in general body size.

*Crosses between strains:* The selected and unselected strains were intercrossed after selection in the Pobla de Lillet and the second Crianlarich series and reared along with the parent strains; generally ten females from each of five cultures were scored. Previous work (ROBERTSON and REEVE 1954a) has shown that crosses between such selected strains, while roughly intermediate in size, usually depart from the midparent value in the direction of larger size and so it is of some interest to discover how this departure is related to cell size and number. Table 2 shows the differences in wing size in log units between the parent populations, together with the deviations from the midparent value for wing cell size and cell number. There is evidently a strong correlation between wing and cell area in the degree of departure from intermediacy. Averaging the values for the six available crosses

TABLE 2

*The departure from intermediacy in wing and cell size and cell number in crosses between strains: in log units*

| Differences between parent strains | Wing area | Wing area | $F_1$—midparent Cell area | Cell number |
|---|---|---|---|---|
| Crianlarich | | | | |
| U × L | 0.029 | 0.010 | 0.006 | 0.004 |
| U × S | 0.103 | 0.024 | 0.024 | 0.000 |
| L × S | 0.131 | 0.034 | 0.033 | 0.001 |
| Pobla de Lillet | | | | |
| U × L | 0.055 | 0.009 | 0.016 | —.007 |
| U × S | 0.054 | 0.022 | 0.028 | —.006 |
| L × S | 0.109 | 0.029 | 0.019 | .010 |
| Average | | 0.021 | 0.021 | 0.000 |

U—unselected strain.
L—large strain.
S—small strain.

gives the same figure of 0.021 for both dimensions. Hence the departure from intermediacy in wing size is characterized by the relatively larger cell size of the crosses. Thus cell number behaves in additive fashion—on a log scale—whereas cell area apparently shows heterosis, which varies according to the type of cross and which is sufficient to account for the departure of wing area from the mid-parent value.

*The Pacific strains:* After five generations of selection in either direction, the four possible crosses between a large and small strain were compared with the parent strains and the unselected stocks. Thorax size was also recorded in these tests, and has been expressed in terms of log squared length, to provide a more suitable scale for comparison with differences in wing area. Table 4 shows the differences between the series in terms of average deviations from the unselected stock. The main points of interest are as follows:

(i) Although selection has led to a striking difference in thorax and hence general body size, the changes in wing size are proportionately less. This is evident in the end column of Table 3 which shows the wing/thorax ratio for the different series (log wing area—log squared thorax length) and is brought out clearly in Figure 1 in which the regression of log wing area on log squared thorax length is fitted to the mean values of the different groups. Although there is virtually complete ge-

TABLE 3

*Selection for body size in Pacific population: in log units*

| Strains | | Squared thorax length | Deviations from unselected | | | Wing—thorax area |
|---|---|---|---|---|---|---|
| | | | Wing area | Cell area | Cell number | |
| Large | A | 0.032 | 0.016 | —0.009 | 0.027 | 0.489 |
| | B | 0.088 | 0.027 | 0.015 | 0.012 | 0.444 |
| Small | A | —0.072 | —0.018 | 0.007 | —0.025 | 0.559 |
| | B | —0.060 | —0.007 | —0.002 | —0.005 | 0.558 |
| Unselected average | | 9.390±.010 | 9.895±0.004 | 0.557±.010 | 9.338±.010 | 0.505 |

TABLE 4

*Crosses between selected Pacific strains (log units)*

| Cross | $F_1$—midparent | | | |
|---|---|---|---|---|
| | Thorax area | Wing area | Cell area | Cell number |
| AL × AS | 0.059 | 0.020 | 0.027 | —0.007 |
| AL × BS | 0.032 | 0.016 | 0.034 | —0.018 |
| BL × AS | 0.022 | 0.012 | 0.019 | —0.007 |
| BL × BS | 0.018 | 0.002 | 0.030 | —0.028 |
| Average | 0.033 | 0.012 | 0.028 | —0.016 |

A and B refer to different large (L) and small (S) strains.

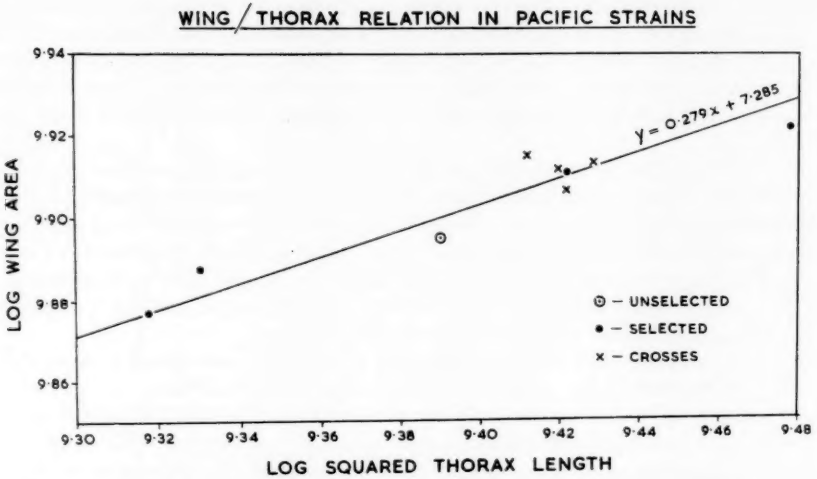


FIGURE 1.—The allometric relation between wing and thorax size in Pacific strains.

netic correlation between the two dimensions, a unit change on the thorax scale is accompanied by only 0.3 unit change in wing size. This particular allometric relation is unexpected since previous experience of selection, the comparison of wild populations (in the preceding paper) and the effects of the alteration of body size by nutritional means would lead us to expect a regression line fairly close to unity.

(ii) The changes in wing size produced by selection are correlated with changes in cell number. Thus the average deviation from unselected for cell size works out at 0.003 and 0.002 log units for the pair of large and small strains respectively, and so the evidence from these selection experiments falls into line with the data from the other tests described in Table 1.

(iii) The crosses between the large and small strains introduce a new feature. All the crosses exceed the unselected in body size and the wing/thorax ratio conforms to the same allometric relationship as the parent and control strains. Thus, whereas an increase beyond the normal level of body size by selection leaves average cell size unchanged, a comparable increase, when encountered in a cross between strains, is associated with an increase in cell size. Judged by the corresponding deviations from unselected, thorax and cell area behave very similarly, and the average deviation works at about the same value of 0.03 log units for both. With such an increase in cell area, the allometric relationship can only be preserved with a proportional reduction of cell number, as is indeed the case.

The relation between the deviation from intermediacy in crosses and the behavior of cell size and number may be considered next (Table 4). As might be anticipated from Table 4, the positive deviation from intermediacy for wing

area is associated with a proportionately greater increase in cell area and, of course, an inverse change in cell number unlike the crosses considered in Table 3, where log cell number was intermediate. It seems likely that the latter situation is typical of crosses between strains with the usual allometric relationship between wing and thorax. With this qualification in mind the association between deviation from intermediacy and relative increase in cell size stands out as the most striking property of crosses between strains.

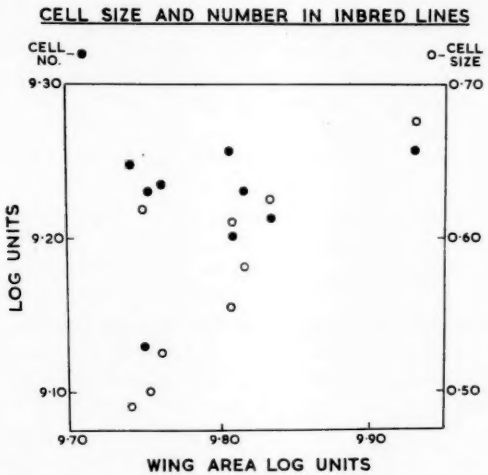


FIGURE 2.—Cell size and number in inbred lines derived from unselected wild populations.

*The comparison of inbred lines*

Data are available for a number of inbred lines, derived from several different populations, and which have been used in other experiments not dealt with here. Although the lines were not all reared at the same time, they were all cultured at 25°C and, since cell size is comparatively unaffected by nutritional variation, comparisons between lines are valid. In Figure 2 average log cell area and log cell number are plotted against log wing area. The differences of wing area are highly correlated with differences of cell area, and there is little evidence of systematic trends in cell number. This is just the reverse of the situation encountered in the study of differences between wild populations, considered in the preceding paper, and also differs from the effects of selection on wing and body size. Prolonged inbreeding evidently destroys the basis for the stability of cell size in wild populations and inbreeding decline appears to be primarily associated with a decline in cell size.

*Crosses between inbred lines*

Another situation has been examined by crossing inbred lines, derived either from unselected stocks or strains selected for large or small body sizes; only lines derived from the same foundation population were intercrossed. The parent lines were all cultured at the same time and eight females were measured from each of five replicated cultures. The symbols I, R, E and C refer to the origin of the lines from the original foundation population: Ischia, Renfrew, Edinburgh and Crianlarich; the subscripts L and S or a numeral refer to their deviation from selected large or small strains or the unselected populations.

The deviations from the midparent value are listed in order of diminishing magnitude in Table 5. On the average, changes in cell area contribute more to the observed heterosis than change of cell number, since the mean deviation works out at 0.049 units for cell area compared with 0.019 for cell number. Also the regression of wing area deviation on cell area deviation works out at $0.71 \pm 0.29$ compared with $0.49 \pm 0.49$ for the regression on cell number deviation. In several instances, especially in crosses between the unselected lines and lines derived from large strains, the $F_1$ deviation is almost entirely reflected in the disproportionate increase in cell size.

This relation between heterosis and cell size recalls the behavior of the crosses between the strains which have been considered earlier. There is a new feature in that log cell number also tends to exceed the intermediate value. There is no instance of a negative deviation, as in the Pacific strain crosses, and this supports the hypothesis that such a deviation is directly related to the peculiar allometric relationship between wing and thorax in these strains. The tendency for cell number to show a positive deviation may be especially characteristic of crosses between highly inbred lines. In the crosses between the Crianlarich and Pobla de Lillet strains, in which average log cell number was intermediate, the parents were highly heterozygous and it may be assumed that three generations of mass selection are unlikely to produce much disturbance in the original genetic equilibrium.

TABLE 5

*Deviation from midparent value in crosses between selected and unselected inbred lines; in log units*

| Cross | Wing area | Cell area | Cell number |
|---|---|---|---|
| $E_3 \times E_S$ | 0.091 | 0.061 | 0.030 |
| $C_2 \times C_S$ | 0.089 | 0.077 | 0.012 |
| $R_2 \times R_L$ | 0.077 | 0.068 | 0.009 |
| $C_S \times C_L$ | 0.075 | 0.033 | 0.042 |
| $E_3 \times E_L$ | 0.066 | 0.052 | 0.014 |
| $I_L \times I_S$ | 0.065 | 0.025 | 0.040 |
| $C_2 \times C_L$ | 0.055 | 0.060 | −0.005 |
| $E_S \times E_L$ | 0.022 | 0.014 | 0.008 |
| Average | 0.068 | 0.049 | 0.019 |

*Aberrant genotypes*

It is clear now that genetic differences in wing size arrived at in different ways, are associated with characteristic changes in cell and/or number. To throw further light on these interrelations between development and genetic behavior genotypes have been created, which may be regarded as aberrant in the sense that they represent arrays of genes unlikely ever to be encountered in wild populations. In the first of the two series of experiments a long inbred line ($E_3$) derived from the Edinburgh population had the third pair of chromosomes replaced by different homozygous or heterozygous pairs of chromosomes drawn from the Renfrew population; four such homozygous and two heterozygous combinations are available for comparison with the line $E_3$; eight females from each of five cultures for each genotype were scored as usual. These genotypes were prepared by Dr. E. C. R. Reeve for other purposes, and I wish to thank him for making them available.

The average values of wing and cell size for the different genotypes are plotted in Figure 3. Two of the homozygous substitutions did not have much effect although wing size is a little smaller; the other two reduced size below the $E_3$ level by respectively six and 12 percent. The two heterozygous substitutions increased size; the differences between $E_3$ and either of these types is highly significant statistically. The line of proportional increase is drawn through the point for $E_3$ and this demonstrates very clearly that the variation in wing area is associated

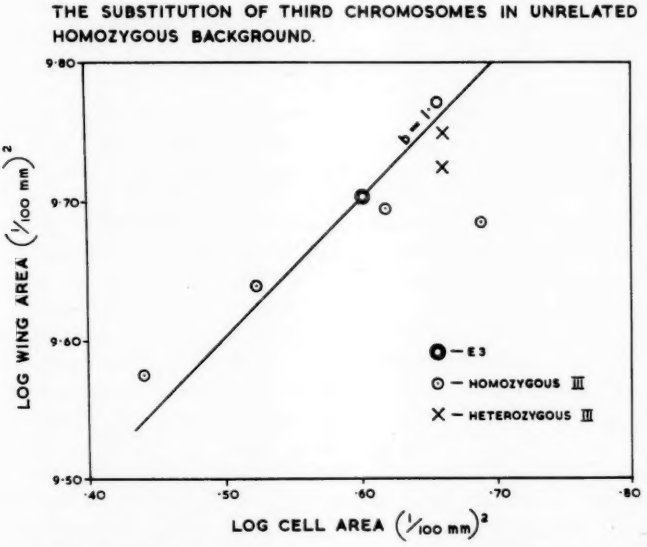


Figure 3.—The effects of replacing the third chromosomes of an inbred line ($E_3$) by homozygous and heterozygous pairs of chromosomes from an unrelated wild stock.

with parallel changes in cell area, only one of the seven points departs much from this line. In addition it is interesting that different homozygous third chromosomes differ so much in effect when substituted in the unrelated genetic background.

In the last series of tests atypical genotypes have been produced by making the third chromosome pair homozygous in a genetic background which could be regarded as representative of a gentically variable wild population. Using the Curly (*Cy*) inversion for II and Moiré (*Mé*) and Moiré Stubble (*Mé Sb*) for III, *Cy Mé* and also *Cy Mé Sb* males were backcrossed for several generations into the wild population, then the *Cy* marker was discarded and the backcrossing continued, using only *Mé* or *Mé Sb* males. A number of such *Mé Sb* males were crossed individually to groups of 8–10 virgin *Mé* females drawn from the backcross stock; these females were divided into two equal groups labelled a and b. *Mé* males and virgin females were collected from the progeny of each male and these carried the same wild third chromosome originally present in the *Mé Sb* parent. The *Mé* males of group a were crossed to the *Mé* females of group b and *vice versa*, to diminish the risk of inbreeding in the genetic background; and, as far as possible, equal numbers of eggs from the two groups were set up together in replicated cultures. The resulting wild type progeny were scored and these provide estimates of the effects of making III homozygous but keeping the rest of the genotype about as variable as is normally the case in flies of the wild population. Such experiments have been carried out on both the Gabarros and Pacific populations. In the former, there was little or no evidence of a tendency for size to decline when the third pair of chromosomes was made homozygous, and so the data are not of much interest in the present context. In the Pacific population, however, a decline in size was more evident although not especially striking. The comparatively small average effect of making the longest pair of chromosomes homozygous, in an otherwise highly heterozygous background recalls the results of earlier experiments in which a variety of homozygous and heterozygous combinations were compared by interchanging chromosomes between lines (ROBERTSON and REEVE 1954b), and emphasises the extent to which heterozygous can be replaced by homozygous combinations before body size is affected. A number of lethals were encountered, especially in the Pacific tests.

The data are set out in Table 6 in terms of the average deviations of wing area from the control wild flies, together with estimates of variance and covariance of wing and cell size; the components are multiplied by $10^6$ to avoid the confusing succession of zeros. In the experimental series wing size ranges from a four percent increase to a 13 percent decrease, compared with the controls. A general picture of the effects of making III homozygous is provided by comparing the pooled average components for the experimental and control series; variance estimates refer to pooled within-culture effects. The third chromosome is the largest and accounts for some 40 percent of the metaphase length of the chromosomes in females (GOWEN 1952). Assuming that the contribution of different chromosomes to total variance is roughly proportional to their length, an appreci-

TABLE 6

*The effects of making the third pair of chromosomes homozygous in the Pacific population*

| Series | Dev. from controls: wing area | d.f. | $\sigma^2_C$ | $\sigma^2_S$ | $cov_{SC}$ | $\sigma^2_N$ | $b_{SC}$ |
|---|---|---|---|---|---|---|---|
| 1 | 0.0414** | 30 | 450 | 1350 | 350 | 1100 | ..... |
| 2 | —0.0162 | 12 | 1420 | 1070 | 52 | 2390 | ..... |
| 3 | —0.0203 | 19 | 170 | 1800 | 740 | 490 | ..... |
| 4 | —0.0273* | 23 | 470 | 2020 | 330 | 2160 | ..... |
| 5 | —0.0462** | 29 | 570 | 3990 | 1130 | 3430 | ..... |
| 6 | —0.0513** | 11 | 1840 | 3130 | —20 | 5010 | ..... |
| 7 | —0.1356** | 41 | 2070 | 3030 | 1440 | 2220 | ..... |
| Pooled average | | 165 | 1010 | 2470 | 750 | 1980 | 0.75±13* |
| Control wild flies | | 93 | 2170 | 2060 | 480 | 3270 | 0.22±10* |
| | | | "Genetic" effects | | | | |
| Experimental | | | 490 | 1760 | 750 | 760 | 1.53 |
| Controls | | | 1650 | 1340 | 480 | 2150 | 0.29 |
| | | | Partition of cell size variance | | | | |
| | | | "Independent" | | "General" | Total | |
| Experimental | | | 170 | | 320 | 490 | |
| Controls | | | 1480 | | 170 | 1650 | |

C, S and N refer to cell and wing area and cell number. Variance components are multiplied by $10^6$ and rounded off to nearest 10.
* Indicates the difference significant at 0.05 level of probability.
** Indicates the difference significant at 0.01 level of probability.

able reduction in variance is expected for additive genetic effects when III is made homozygous. There is clear evidence of this in the variance estimates for both cell area and cell number which are reduced from respectively 2170 and 3270 units to respectively 1010 and 1980 units. But the variance of wing size is not reduced and is actually somewhat higher in the experimental than the control series. There is also a distinct suggestion that as average wing size declines so does its variance increase.

It has been shown in the preceding paper that genetically different individuals of the same wing size may differ in cell size and number, and that genetic effects which result in such an inverse relation between cell size and number make an appreciable contribution to the total phenotypic variation of either in wild populations. The loss of genetic variance effected by making III homozygous will reduce variation in this respect, and this is probably the main reason for the observed reduction in the variation of cell size and number. But at the same time, the variation of wing size has not declined as well, while the covariance between wing and cell size has increased. This suggests that the presence of a homozygous pair of third chromosomes has reduced the stability of wing size and has led to an increase in the relative importance of genetic effects which influence wing development in a characteristic way and that this accounts for the increased covariance between wing and cell size. Apart from series 6, which has few degrees of freedom, there is a general tendency for the covariance to increase as

wing size declines.Thus the evidence from this test falls nicely into line with the other data.

The analysis can be taken a little further by estimating purely genetic effects from the differences between components of total variance with corresponding estimates of environmental variance. For the latter we can use the estimates derived from the within-culture variance of the genetically uniform crosses between inbred lines, quoted in the preceding paper (ROBERTSON 1959). Although these were considered valid for wild flies, they probably underestimate the effects of environmental variation in the present instance, since the general sensitivity to environmental variation is likely to be higher than average in the subpopulations. However, such differences are probably secondary to the contribution of genetic variation to the total phenotypic variance. After subtracting the estimated environmental variance, the estimates of genetic variation of cell size work out at 490 and 1650 units for experimental and control series respectively, while for wing size the corresponding estimates are 1760 and 1340. The genetic regression of wing on cell size is estimated as 1.53 and 0.29 for the two series. These estimates can be used to partition the total genetic variation of cell size into what may be termed, purely for convenience, "general" effects which are responsible for a correlation between wing and cell size and "independent" effects i.e., variation in the inverse relations between cell size and number. Too much reliance cannot be placed on the figures arrived at in this way, since they involve a number of assumptions which are probably only partly justified. At least they serve to make a relevant point.

It appears that the variance of the so-called "independent" effects is drastically reduced by making III homozygous, in an otherwise normal genetic background, but the contribution of "general" effects to cell size is increased. Hence the composition of the variance of cell size in experimental and control series is different —apparently 65 percent of the variance of the former is associated with wing size, compared with a mere ten percent in the latter. Hence, in spite of the outward similarity of total genetic variance of wing size in the two series, the nature of this variation differs with respect to the development of the wing. In the wild stock most of the genetic variance of wing size is associated with cell number, while in the experimental series, the predominant association is with cell size and this is clearly related to the increased effects of segregation, which, in turn, is a direct consequence of the atypical gene arrays produced by fixing different third chromosomes.

## DISCUSSION

It was shown in the preceding paper that the manner in which growth is apportioned between cellular growth and cell proliferation varies according to environmental conditions. The present paper has demonstrated that this is also true of different kinds of genetic change since the cell size and number relations evince a characteristic pattern related to the way the differences in wing or body size are produced. Since cell size and number are dual aspects of growth, dynami-

cally interrelated and subordinated to hormonally regulated processes which control final body mass, change due apparently to only one or other type of change should be regarded as a property of special conditions, both genetic and environmental, rather than as evidence for genetic independence of cell size or cell number. It remains for future work to specify these conditions more precisely, but there is now sufficient evidence to set down some of the rules of behavior of cell size and number in the wing of *Drosophila melanogaster* at 25°C, and point to relations between the genetic behavior of differences in wing and body size and the cellular make-up of the wing.

The comparisons between a series of wild populations, described in the earlier paper, showed that the average cell size in the wing is similar in different populations, so that differences in wing size are expressed by differences in cell number. Selection for large and small body size in four of these wild populations showed that striking differences in wing size left average cell size virtually unchanged. Also when larvae are reared on media deficient in some essential nutrient there is a well-marked tendency for smaller body size to be reflected in a reduction of cell number in the wing, unless the conditions are too unfavorable when cell size is reduced as well. Hence a general feature of wild population is the lability of change via cell number, coupled with stability of cell size, in the presence of both nutritional variation and the genetic changes produced by short term selection for large and small body size.

There is nevertheless considerable variation in the observed wing cell size, which has been demonstrated by comparing the variance components derived from wild flies with the corresponding estimates for genetically uniform crosses between inbred lines (ROBERTSON 1959). Such variation presents two aspects. Firstly, genetically different individuals of the same wing size may differ in cell size and number, and selection for large and small cells showed that it was possible to shift the cell size and number quite strikingly without affecting wing size. It was suggested that such variation in the inverse relations between cell size and number is tangible evidence of genetic differences in growth which are regulated to ensure stability of final body or wing size.

Secondly, although the estimates suggested that most of the genetic variation of wing size is correlated with variation of cell number—since confirmed in the selection—there is also an appreciable genetic correlation between wing and cell size, which results in a genetic regression of wing on cell area of 0.3 log units. Since no evidence of parallel change in cell size accompanied the changes in wing size due to two-way selection, this covariance is nonadditive in behavior. The effects of crossing the selected strains provide a valuable clue. It will be recalled that crosses between the various Crianlarich and Pobla de Lillet strains produced an $F_1$ which exceeded the midparent value by an amount which could be attributed to the relatively larger cells, while cell number was intermediate on the log scale. In the other tests with the Pacific strains, all the crosses between the large and small strains exceeded the level of the unselected population and all had larger cells. Thus increase in wing size by selection was associated with change

in cell number, but a comparable deviation above the normal size in the crosses was associated with change in cell size. The deviations from intermediacy in the Pacific crosses showed fair agreement between wing cell area and squared thorax length, but cell number declined below the intermediate level. This was attributed to the unusual allometric relationship between wing and thorax size in these strains, and the relations found in the other crosses were considered more typical.

The $F_1$ deviation from intermediacy in wing area could be attributed either to:

(a) a general tendency to dominance of larger over smaller size, or

(b) the creation in the $F_1$ of a novel gene combination with the property of increasing wing size in a characteristic way i.e., by increase of cell size.

If, (a) is correct it seems a strange coincidence that the dominance deviation should tally so closely with the deviation for cell area, instead of being reflected in cell number as well, especially as the parent strains differ in cell number. Hence the second hypothesis is preferred. If interactions of this sort also occur among the gene arrays of the normal wild population, although on a less dramatic scale, this could account for the nonadditive genetic correlation between wing and cell size.

So far the most effective way of increasing body and cell size together is by crossing strains in which one or both parents have been selected for large or small size. Hence gene combinations can be created which involve developmental changes which differ from those which are associated with selection for larger body size. The level of heterozygosity in crosses between large and small strains will be higher than in the unselected population, but this effect is probably due to the particular changes which have accompanied the selection of the parental strains rather than merely to increased heterozygosity in the $F_1$. The nature of these changes is being studied in further experiments.

It may be inferred from the genetic analysis of body size, described in earlier numbers of this series, that nonadditive effects on body size are minimal in a population in equilibrium and that disturbance of the equilibrium by selection, inbreeding or other means is likely to increase the relative contribution of nonadditive effects to the total genetic variance. If such interactions are especially associated with developmental changes which are finally expressed by parallel changes in wing and cell area, factors which disturb the equilibrium or balance (MATHER 1954) will result in greater apparent determination of wing by cell size.

Support for this inference is provided by the effects of making III homozygous in the genetic background of the Pacific population. In spite of the inevitable loss of genetic variation occasioned by making the major chromosome homozygous—and probably demonstrated in the decline in the variation of the inverse relations between cell size and number—there is no decline in the variation of wing size, compared with flies of the wild population. This must be due to an increase in the relative importance of nonadditive interaction which follows upon the fixation of the novel and presumably unfavorable gene arrays in the subpopulations. Such

effects are associated with a striking increase in the covariance between wing and cell size, and there is a general tendency for the covariance to be higher in the subpopulations with the smaller average wing size.

The highly abnormal genotypes created by substituting different third chromosomes from one strain in the homozygous background of an unrelated inbred line lead to considerable differences in wing size which were closely associated with parallel changes in cell size. The unselected inbred lines follow the same pattern since the regression of log wing area on log cell areas is close to unity. They make a striking contrast with the comparisons between wild populations in which differences of wing size were correlated with cell number. Inbred lines may be classified as abnormal genotypes, compared with the gene arrays which are present in an outbreeding species.

The heterosis in crosses between inbred lines—which represents a return to more normal gene arrays—is expressed by relative increase in cell area and to a lesser degree by increase in cell number, suggesting that the decline in wing size due to inbreeding is particularly associated with parallel changes in cell size. Naturally this general trend may be partly obscured by random fixation of genes which influence the inverse relations between cell size and number.

It has been noted above that the genetic correlation between wing and cell size in wild populations is nonadditive in behavior. Selection for large cell size, failed to effect a permanent increase in wing size, whereas selection for small cells lead to a decline in wing size. In the earlier paper (ROBERTSON 1959) it was shown that an eight percent difference in body size, produced by selection for small cells, behaved as entirely recessive in a backcross to the unselected stock. Previous experience and unpublished data relating to the genetic behavior of comparable differences due to a few generations of selection for small body size—as opposed to small cell size—has indicated more nearly additive behavior in such crosses. Evidence for such recessive or hypostatic behavior has been detected in strains subjected to prolonged selection (ROBERTSON 1954). Since genetic changes which influence the stability of body size in a population—as measured by the level of phenotypic variation due to segregation and recombination—are more likely to be associated with changes in cell size rather than number, the reduction of body size by selecting for smaller cells apparently affords a direct way of destroying the essential conditions which underlie the stability of body size. Possibly gene combinations are favored which lower the capacity for regulation during growth. Short-term selection for smaller body size is much less effective in this respect. Thus different criteria of selection may lead to an equivalent reduction of body size by different kinds of developmental change which are also distinguished by characteristic differences in genetic behavior.

The coexistence of genetic variation and comparative phenotypic stability is a widely recognized property of outbreeding species (MATHER 1954). In a wild population the essential conditions for "normal" growth are present in most individuals and the statistical evidence for epistasis in relation to body size is secondary to the additive effects which provide the basis for selection response.

From the analysis presented in earlier papers of this series (ROBERTSON 1954; ROBERTSON and REEVE 1954b; ROBERTSON 1955), it may be inferred that genetic changes which disturb the equilibrium, especially after prolonged selection are also responsible for greater evidence of epistasis. Body size behaves in some respects like a character which varies about an intermediate optimum and, in others, like one held to an upper level. The latter property derives from the genetic conditions which favor stability of body size in a genetically variable population. Genetic changes which lead to increased effects of segregation and recombination—most commonly, but not inevitably, associated with inbreeding —also tend to reduce body size so that there is an inverse relation between mean and variance. They will also involve an increase in the covariance between wing and cell size, which provides a sort of empirical index of the loss of balance or coadaptation, to use different terminologies. In such situations, gene combinations which tend to repair the damage to the pre-existing capacity for regulation of growth will lead to larger body size, and they will behave as epistatic over gene combinations which are less effective in this respect. Often such interaction looks like simple dominance, but when analysed, generally turns out to depend on the joint action of a number of genes. Thus the stability of body size rests on epistatic interaction which minimizes the effects of segregation on key developmental processes.

It follows that a high level of additivity, associated with variation of cell number, is only possible if these key processes are comparatively unaffected by segregation and recombination, otherwise epistatic effects will dominate the genetic variance, as is probably the case in the subpopulations created by making the third chromosome pair homozygous. Hence the property of additive genetic behavior on body size is conditional on the rest of the genotype and there are no grounds for gratuitous speculation about the primary effects of the gene differences in question.

It is clear therefore that the characteristic differences in the cellular make-up of the wing can be reconciled with the conceptual scheme founded on purely genetic methods of analysing the variation of wing and body size. This is encouraging since it suggests fresh experimental approaches to the study of genetic variation in populations and offers the prospect of establishing further interrelations between the statistical properties of variation and development.

## SUMMARY

1. The relations between cell size and number in the wing of *Drosophila melanogaster* have been studied in different genetic situations which involve differences in wing and body size. Estimates of cell size and number are derived from the cell density in the central region of one surface of the wing. Details of procedure have been given in the preceding paper of this series.

2. Short-term selection for large or small body size in several different wild populations was accompanied by parallel changes in cell number, and average cell size in large and small strains was about the same.

3. When selected large and small strains from the same population were intercrossed or backcrossed to the parent strains, $F_1$ wing size exceeded the midparent value, especially in crosses between strains selected in the opposite direction. The average departure from intermediacy for log wing and log cell area was the same and log cell number was intermediate. In a series of crosses between large and small strains derived from another population the deviation from intermediacy for log squared thorax length and log cell area showed good agreement.

4. Such heterosis which influences development in this characteristic way is attributed to interaction arising from the novel gene combinations created in the $F_1$ of such crosses. Similar interaction, of lower order of magnitude, probably accounts for the low genetic correlation between wing and cell size which is regularly found in wild populations and is consistent with the nonadditive properties inferred from the average constancy of cell area when large and small strains are created by selection.

5. The regression of log wing area on log cell area for a number of inbred lines was close to unity.

6. The heterosis in crosses between inbred lines derived from selected as well as unselected lines was predominantly associated with relative increase in cell area, and to a lesser degree with increase in cell number.

7. Cell size and number relations were studied in an array of genotypes in which homozygous and heterozygous pairs of third chromosomes from a wild population were substituted in the background of an unrelated inbred line. Most of the considerable variation in wing area between different types was expressed by change in cell area.

8. Another genetic situation was examined by making a number of third chromosomes homozygous in the otherwise normal, genetically variable background of a wild population. The expected substantial loss of variation was reflected in a drastic reduction in the variance of cell size and number, which could be partly attributed to lower variance of the inverse relations between cell size and number, which appear to be independent of total wing size. But the variance of wing size was just as great as in the wild flies. Also the covariance between wing and cell size was greatly increased in the experimental series. Thus although the variance of wing size was about the same in control and experimental group, in the former variation in cell size was relatively unimportant compared with variation in cell number, whereas in the latter the situation was reversed. The disturbance of the genetic equilibrium due to making III homozygous evidently increased the effects of segregation and recombination in the genetic background—due to greater contribution of nonadditive effects—and this was associated with increased covariance between wing and cell area.

9. The data indicate that equivalent deviations of wing and body size from the average size of a given population may be achieved by different kinds of developmental change which are in turn associated with characteristic differences in

genetic behavior. Put briefly, changes which are expressed by alteration of cell number behave more or less additively whereas changes due apparently to alteration of cell size are nonadditive.

10. The data are discussed with reference to the findings described in the preceding paper of this series. It is suggested that the processes which underlie the association between wing and cell size play a key role in maintaining the stability of wing and body size in a genetically variable population. Gene arrays which make for higher stability are epistatic over those which are less effective in this respect. Genetic changes which interfere with such processes increase the covariance between wing and cell size, and, at the same time lead to an increase in the contribution of nonadditive effects to the total variance.

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# THE GENETICAL ANALYSIS OF CHARACTERS WITH CONTINUOUS VARIABILITY ON A MENDELIAN BASIS. I. MONOHYBRID SEGREGATION

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EVERY typical Mendelian segregation is based on an *alternative or qualitative variability*. This applies to characters controlled by one or a few pairs of alleles clearly following the Mendelian laws, each with a pronounced individual effect. These individuals as carriers of the characters concerned can be divided into clearly separate classes; hence an analysis of the separating units (genes) in the segregating generations no longer presents any fundamental difficulties.

The position is different in the case of characters with *continuous or quantitative variablity*. Here the individuals as character carriers cannot be divided into clearly separate groups; they differ only with regard to the degree in which the character is pronounced, so that instead of groups there are numerous transitions. In the segregating generations there is no clear Mendelian segregation into definite phenotypical groups corresponding to the individual genotypes (RIEGER and MICHAELIS 1958).

As continuous variability does not permit a simple genetic analysis by Mendelian methods, preference was at first given to consideration of the frequency distributions of the parents and their filial generations. This led to an estimation of the number of genes and of dominance relations as well as to the study of the interaction of polygenes.

It is not intended here to deal any further with the various papers in the field of quantitative inheritance, as they have been discussed in a series of papers, e.g., recently by NEI (1959). Instead, it will be shown by a simple example that a genetic analysis of characters with continuous variability can be achieved by means of discriminant function (WEBER 1957).

First some characteristic differences in the description of both kinds of characters:

In the description of characters with alternative variability, the total impression on our senses is expressed by a certain term, e.g., early or late ripening, red or white color of flower; whereas a character with continuous variability is only inadequately described by the data of one measurement. In the character "leaf size", for instance, the length gives no indication of the area or width of the leaf. In addition, these data have dimensional designations, i.e. a dimension in the physical sense, and variance within a phenotypic group. The characteristics just mentioned—physical dimension and variance—are not entailed in the terms describing characters with alternative variability.

Just as it follows from the disparity between these characteristics that a genetical analysis of characters with continuous variability by Mendelian methods is impossible, so their contrast also suggests qualifying in some way the dimensional data of a character with continuous or quantitative variability, in so far as its genetics are to be treated on a Mendelian basis. This will now be specified.

As a method of qualifying these test data, the total measurement of discriminant function (WEBER 1957) is applicable; its properties are similar to, or closely resemble those of the terms used for the description of characters with alternative variability.

The onesidedness of the description of characters with continuous variability by one measurement only, can be avoided by describing the character in question, e.g. leaf size, by several single measurements, e.g. length, width and area of the leaf. If these single measurements are combined to a total measurement by means of discriminant function, then this numerical total measurement will correspond to the total impression of a character with alternative variability, expressed by one term. This total measurement of discriminant function has no dimensional designation; it is without dimension in the physical sense, like the term red or white color. Variance of the total measurements within a phenotypic group is indeed still present, but by means of maximization of the ratio of variance "between the groups" to "within the groups," it will tend to approach zero. As a consequence, with respect to variance the total measurement of discriminant function is also very similar to the terms describing characters with alternative or qualitative variability.

The different methods of discriminant analysis as well as their application to genetics and plant breeding are specified in a collective review by WEBER (1957), hence they are not dealt with again here. As the application of discriminant analysis has so far not led to a clear Mendelian segregation, e.g., in the paper of K. MATHER and U. PHILIP in MATHER (1949), the following simple example will show how the qualification of quantitative data by discriminant function leads to typical Mendelian segregation in the case of characters with continuous variability in the $F_2$ generation.

### *Monohybrid segregation of the character cotyledon size in* Lycopersicum esculentum *Mill.*

The inheritance of cotyledon size was tested quantitatively in two varieties of tomato. The normal variety "Bonner Best" (=B) was crossed with the dwarf variety "Professor Rudloff" (=R). B has a larger cotyledon than R. B's larger cotyledon is linked with a longer hypocotyl and the smaller cotyledon of R with a shorter hypocotyl (See Figure 1). B's larger cotyledon cannot be distinguished with certainty from R's smaller one on a purely qualitative basis. The hypocotyls of R and B are qualitatively easy to distinguish owing to obvious differences in length so that the hypocotyl lends itself as a controlling factor within the meaning of KAPPERT (1953).

The seeds of the parents, $F_1$ and $F_2$ were sown singly into seed boxes at distances

FIGURE 1.—The average sizes of hypocotyl and cotyledon of the parents, R (left) and B (right).

of 3 cm × 3 cm. Four weeks later the seedlings were taken out. Sixty plants each of the parents R and B and of $F_1$ were taken at random, whereas all of the 526 $F_2$ individuals were taken. One cotyledon and the hypocotyl of each plant were cut off, pressed and kept in a herbarium. From a qualitative analysis based on the controlling factor hypocotyl length, it could be seen that the $F_1$ was similar in phenotype to parent B. The $F_2$ showed segregation at a ratio of 3:1 (397 individuals having long and 129 short hypocotyls = 3:0.975; $\chi^2 = 0.063$; P = 0.80). Figure 2 shows the frequency distributions of the character hypocotyl length for the parents and $F_1$. Figure 3 shows the same for $F_2$.

*Gene analysis on the basis of several single measurements of the cotyledon*

The total length, greatest width and area of the above mentioned cotyledons and the hypocotyl length ($=x_1$) were found. The leaf itself is described by two single measurements: $x_2$ = the quotient of length by width of the cotyledon, $x_3$ = the area of the cotyledon.

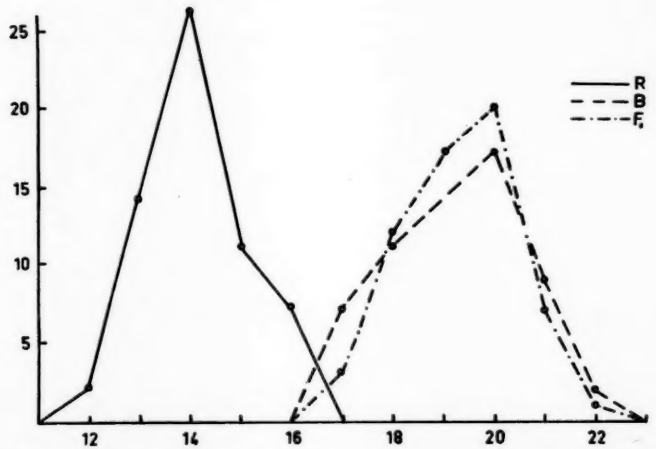


FIGURE 2.—Frequency distributions of the controlling factor hypocotyl length ($=x_1$) for the parents and $F_1$.

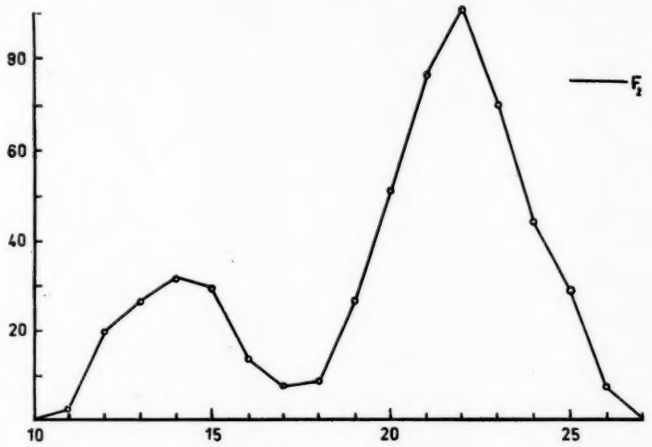


FIGURE 3.—Frequency distribution of the controlling factor hypocotyl length ($=x_1$) for $F_2$.

Figures 4a and 4b show the frequency distributions of the single measurements $x_2$ and $x_3$ of the parents and $F_1$. From these it may be seen that the measured characters of parent B are clearly dominant, for the distributions of B and B × R overlap almost entirely. It may, therefore, suffice to calculate the discriminant function from one of the two collectives R and B, and R and $F_1$ respectively (WEBER 1957). We shall take the parents R and B.

The coefficients $b$ for the discriminant function $X = b_2x_2 + b_3x_3$ are obtained from the following equation:

$$\begin{aligned} b_2 \text{ var } x_2 \quad &+ b_3 \text{ cov } x_2x_3 = d_2 \\ b_2 \text{ cov } x_2x_3 &+ b_3 \text{ var } x_3 \quad = d_3 \end{aligned}$$

$$\begin{aligned} + 2446.5333\, b_2 + 344.6667\, b_3 &= + 7.6667 \\ + 866.5333\, b_3 &= + 5.9333 \end{aligned}$$

$$\begin{aligned} b_2 &= + 0.002300 \\ b_3 &= + 0.005925 \\ \text{or } X &= + 1\, x_2 + 2.575\, x_3 \end{aligned}$$

$$F = 110\ (n_1 = 2, n_2 = 117);\ \tfrac{1}{2}\ \text{P} = 0.12\ \text{(misclassification)}$$

Figure 4c shows the distributions of the total measurement $X$ for the parents R and B.

*Consideration of the frequency distributions of the single measurements $x_2$ and $x_3$ of $F_2$ with 526 individuals*

Figures 4d and e show the frequency distributions of the single measurements $x_2$ and $x_3$ for $F_2$. The dotted lines represent the distributions of R and B on the

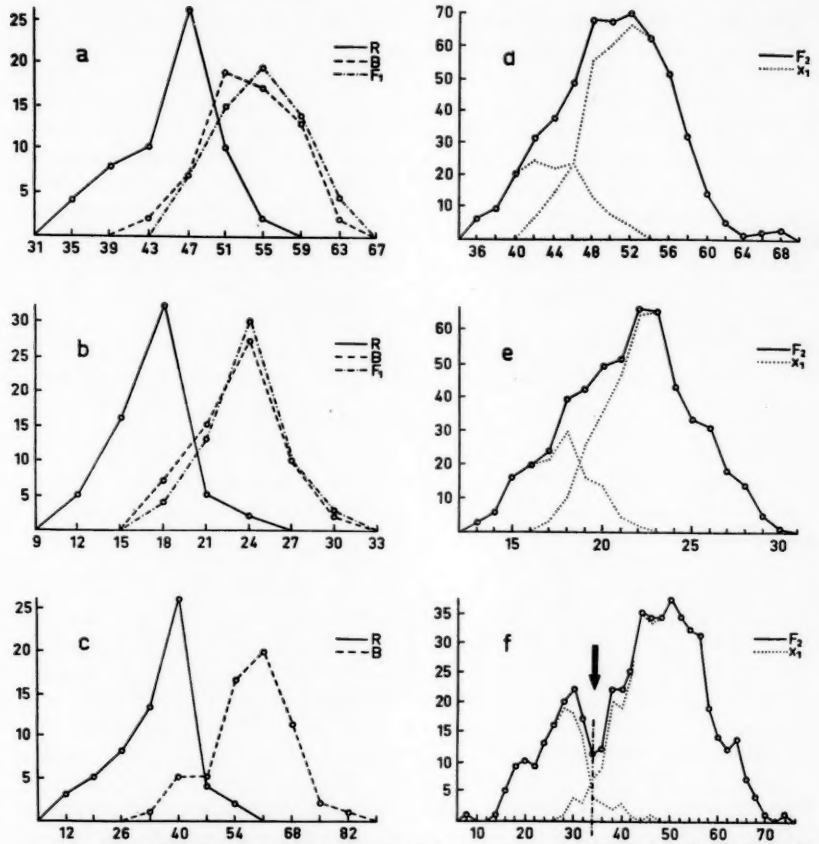


FIGURE 4a.—Frequency distributions of the single measurement $x_2$ (length/width ratio of the cotyledon) for the parents and $F_1$.

b. Frequency distributions of the single measurement $x_3$ (area of cotyledon) for the parents and $F_1$.

c. Frequency distributions of the total measurement $X$ from $x_2$ and $x_3$ for the parents.

d. Frequency distribution of the single measurement $x_2$ for $F_2$.

e. Frequency distribution of the single measurement $x_3$ for $F_2$.

f. Frequency distribution of the total measurement $X$ from $x_2$ and $x_3$ for $F_2$.

(In Figures 4d, e and f, the dotted line represents the distributions based on the controlling factor $x_1$ = hypocotyl length.)

basis of the controlling factor $x_1$. No segregation can be inferred from the continuity of the $F_2$ distribution (solid line). The distributions of the segregation collectives (dotted lines) within the $F_2$ overlap to such an extent that a continuous frequency distribution results. Supposing no controlling factor to be present, then an analysis of the $F_2$ would not be possible.

If, however, the previously calculated discriminant function is applied to the data of the single measurements of the $F_2$, the frequency distribution of their total measurement (Figure 4f) will be notched deeply (see arrow). It no longer shows continuity as do the distributions of the single measurements of the $F_2$ (Figures 4d, e). This notch in the distribution of the total measurement of the $F_2$ clearly indicates that two collectives are present. The other, considerably smaller notches in this distribution are caused by the particularly close grouping. These small notches can be made to disappear by a process of adjustment, but the deep notch remains (Figure 5).

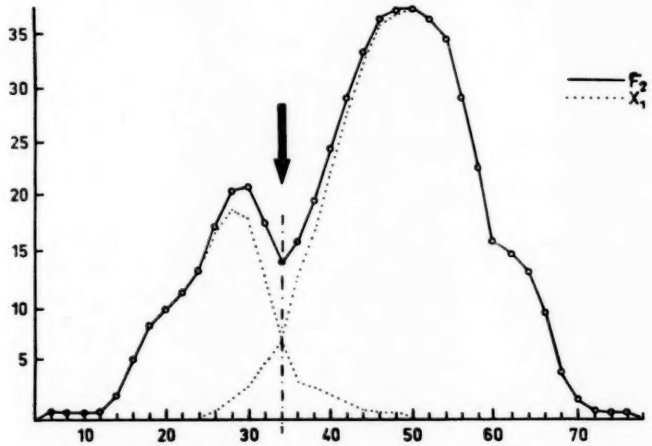


FIGURE 5.—Figure 4f after the adjustment.

The ordinate is drawn through the lowest point of the notch. If this ordinate is regarded as a border line, it will divide the area below the distribution curve of the total measurement of $F_2$ in the ratio of 3:1 (3:0.971).

The concurrence of these segregation results (3:0.975 in qualitative and 3:0.971 in quantitative analysis) shows that the segregation of continuous $F_2$ distributions into their segregational collectives by discriminant analysis is a practicable technique for the gene analysis of characters with continuous variability.

(The very extensive tables of measurements which cannot be printed for technical reasons, may be obtained from the author on request.)

## CONCLUSIONS

According to CHARLES and SMITH (1939), MATHER (1941) and other authors, the isolation of unit characters (monogenic) in quantitative inheritance is practically impossible, as quantitative character variation is supposed to be polygenic.

It has, however, been proved here in a simple way that the size difference between the cotyledons of two varieties of tomato, is caused by one single pair of factors, which means there is monogenic inheritance. (The formula for estimating the least number of genes involved in a crossing indicated 0.258 genes according to SEREBROVSKY (1928). According to the rules of CHARLES and SMITH (1939), there is additive gene action.)

Further examples in WEBER (1958) show that the methods of classical genetics for the analysis of separating units (genes)—including gene localization by means of linkage analysis—are applicable also to quantitative character variation, through segregation of continuous $F_2$ distributions by discriminant analysis.

It may further be of interest that in studying the inheritance of the fruit characters of *Cucurbita pepo*, SINNOTT (1935) found form indices to segregate more markedly than dimensional measures. This is quite understandable, index being a combination of two single but related measurements, very much like the total measurement of discriminant analysis.

BUTLER's (1941) investigations into the genetics of the fruit size of tomatoes showed that ultimate fruit size is determined by a number of factors, e.g. the number of cell divisions before bloom, elongation after bloom, fruit shape (height/width ratio), the number of carpels, etc. Mathematically, this would mean that fruit size ($=X$) is a function of the single measurements just mentioned ($=x_1, x_2, \ldots, x_p$); or $X = f(x_1) + f(x_2) + \ldots + f(x_p)$. This definition (WEBER 1957) leads to representation of the fruit size ($=X$) by a single numerical expression—the total measurement, obtained by combination of the single measurements ($x_p$). If discriminant analysis were applied to the results of BUTLER's investigations, it is quite possible that genetic analysis of these total measurements may lead to Mendelian segregational figures.

In the examples given so far there was complete dominance of the characters of one parent; in conclusion, a model will be demonstrated in which the mean values of the single measurements of $F_1$ are higher than those of the parents (see Figure 6). Recessive epistacy (BATESON 1907) is assumed, whereby the character determined by gene *A* is modified by a second gene, *B* (KAPPERT 1953). Let $P_1 = AAbb$ and $P_2 = aaBB$ represent the parents. Then let $F_1$ be *AaBb*. $F_2$ would then show the following segregation:

| Phenotype | Genotype |
|---|---|
| 9 $F_1$ | 9 *A*—*B*— |
| 3 $P_1$ | 3 *A*—*bb* |
| 4 $P_2$ | 3 *aaB*— and 1 *aabb* |

Figure 6 shows segregation analysis after combination of the single measurements $x_1$ and $x_2$ of a given character.

It might be of interest to examine the problem of heterosis in a similar way.

## SUMMARY

A method of genetical analysis of characters with continuous variability on a Mendelian basis is described and illustrated by a simple example of the inherit-

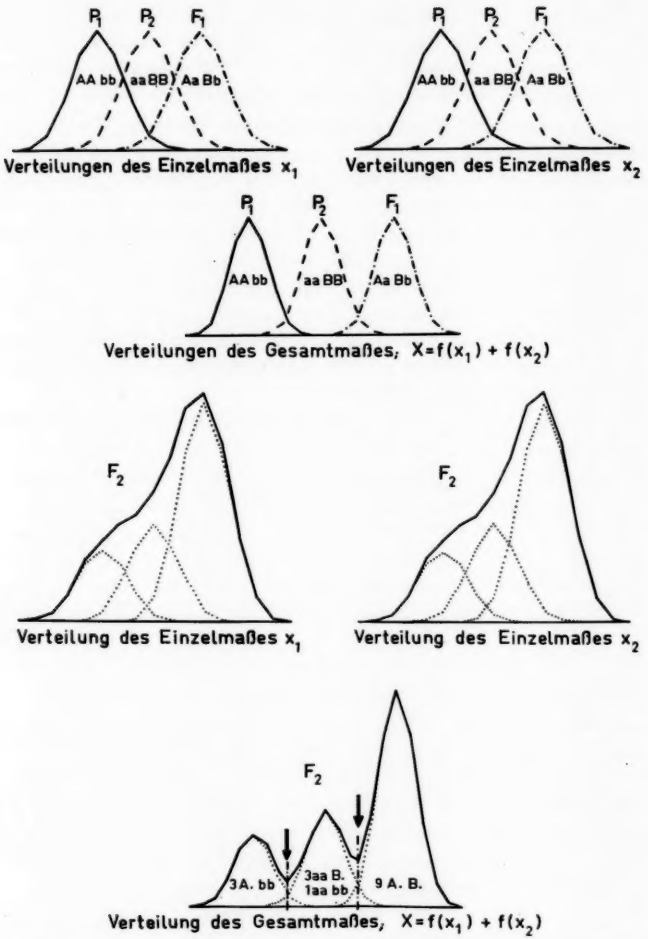


FIGURE 6.—Model of a dihybrid segregation (9:3:4) of a character with continuous variability, based on the total measurement $X = f(x_1) + f(x_2)$ (taken from WEBER 1958).

ance of cotyledon size in two varieties of tomato. The character concerned is specified more precisely by a number of single measurements than by one measurement only. These single measurements are combined to a total measurement by the aid of a discriminant function. The frequency distributions of the single measurements of the $F_2$ generation reveal no segregation whatever owing to their continuity, whereas the frequency distribution of the total measurement of the $F_2$ generation shows a marked notch. From this discontinuity of the frequency dis-

tribution of the total measurement of the $F_2$, the number of segregating collectives as well as their relative shares leading to the Mendelian segregational figures, are to be inferred.

ACKNOWLEDGEMENTS

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# PARTITIONS OF HEREDITARY VARIANCE FOR VARIOUS GENETIC MODELS[1]

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OUR present knowledge of gene action for qualitative characters indicates these actions may be almost as varied as the number of genes, and there is no reason for believing this not to be true for quantitative characters. While transformations would be useful for particular types of gene action (MATHER 1949), they are of doubtful value in realistic situations where many types are probably involved. For this reason, it is helpful to maintain the linear descriptive model, which was proposed earlier (COCKERHAM 1954) and for which the partitions of variance were derived. Then by considering various genetic models, one can see how the hereditary variation is distributed among the various partitions. In this respect the partitioning method serves as a yardstick for comparing genetic models. More important, however, an opinion can be obtained on the power of the yardstick to discriminate among genetic models from experimental data.

More general forms of the models considered by HORNER *et al.* (1955) will be considered here. The partitions of variance will be given, and the models will be examined in terms of the partitions in a very general way.

## *Additive effects between loci*

When gene action is additive from locus to locus, a general model is

$$Y = \sum_i \beta_{ij}, \tag{1}$$

where $i$ goes from one to $s$ (the number of loci). $j$ indicates whether the locus is homozygous dominant, heterozygous, or homozygous recessive and takes values of 2, 1 and 0 for the three respective types. Dominance and recessive are used here to distinguish the two genes, and do not necessarily reflect their action. $j$ takes one of its values for each $i$ or locus; these specify the genotypic value, $Y$. For example, the $Y$ values for two loci are:

| | BB | Bb | bb |
|---|---|---|---|
| AA | $\beta_{12} + \beta_{22}$ | $\beta_{12} + \beta_{21}$ | $\beta_{12} + \beta_{20}$ |
| Aa | $\beta_{11} + \beta_{22}$ | $\beta_{11} + \beta_{21}$ | $\beta_{11} + \beta_{20}$ |
| aa | $\beta_{10} + \beta_{22}$ | $\beta_{10} + \beta_{21}$ | $\beta_{10} + \beta_{20}$ |

(2)

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Since gene action is additive from locus to locus, there are only one factor partitions of the additive and dominance type. When the population is in linkage equilibrium, which is assumed throughout this study, these partitions are given in Table 1. For type of variance $a_i$ is used to indicate the additive variance for

TABLE 1

*Partitions of variance for additive effects between loci*

| Number | Type | Variance* | Total Variance |
|---|---|---|---|
| s | $a_i$ | $\alpha_i A_i^2$ | $\sigma_{10}^2$ |
| s | $d_i$ | $\delta_i D_i^2$ | $\sigma_{01}^2$ |

* $\alpha_i = \dfrac{2u_i v_i}{(1+F)}$, $\quad A_i = (u_i+Fv_i)(\beta_{i2}-\beta_{i1}) + (v_i+Fu_i)(\beta_{i1}-\beta_{i0})$

$\delta_i = \dfrac{u_i v_i (u_i+Fv_i)(v_i+Fu_i)(1-F)}{(1+F)}$, $\quad D_i = (\beta_{i2}-2\beta_{i1}+\beta_{i0})$

the $i$th locus and $d_i$ to indicate the dominance variance for the $i$th locus. This method is useful in extending to joint (two or more) factor partitions (epistasis). For example $a_i \times a_j$ is the additive by additive partition involving the $i$th and $j$th loci. Number refers to the number of partitions for each type which are summed to give the total variance for that type. There are $s$ additive and $s$ dominance partitions, one for each locus. $u_i$ and $v_i$ are the frequencies of the dominant and recessive genes, respectively, and F is WRIGHT's inbreeding coefficient. The partitions of variance for this model and the other models to be considered can be found for two loci by direct substitution in the formulae given by COCKERHAM (1954), where the method of obtaining the partitions for more loci is also given. The partitions of variance for this genetic model have been understood for some time and were presented here primarily to introduce notation and so that partitions of variance of other models may be compared with partitions of this model.

*Multiplicative action between loci*

A model which specifies multiplicativeness is

$$Y = C \prod_i \beta_{ij}, \tag{3}$$

where $C$ is some base quantity which is multiplied by the effects ($\beta_{ij}$'s) of all loci. $\prod$ means to multiply all the indicated terms. For two loci, the various $Y$'s are

| | BB | Bb | bb |
|---|---|---|---|
| AA | $C\beta_{12}\beta_{22}$ | $C\beta_{12}\beta_{21}$ | $C\beta_{12}\beta_{20}$ |
| Aa | $C\beta_{11}\beta_{22}$ | $C\beta_{11}\beta_{21}$ | $C\beta_{11}\beta_{20}$ |
| aa | $C\beta_{10}\beta_{22}$ | $C\beta_{10}\beta_{21}$ | $C\beta_{10}\beta_{20}$ |

(4)

Since the loci are also assumed to be uncorrelated in occurrence, i.e., $f_{ik} = f_i.f_{.k}$ and so on, it is helpful to note that various means are products of means of the effects at each locus. For example, where $P_k$ represents the average of the $\beta_{kj}$'s for the $k$th locus, i.e.,

$$P_k = u_k^2\beta_{k2} + 2u_kv_k\beta_{k1} + v_k^2\beta_{k0} + Fu_kv_k(\beta_{k2}-2\beta_{k1}+\beta_{k0}), \quad (5)$$

then each of the three marginal means for the $i$th locus is given by $\beta_{ij}\, C_{k \neq i} \pi P_k$, and the mean of all the $Y$'s is $C \pi_i P_i$.

The total genotypic variance can be expressed in a compact form as

$$\sigma_Y^2 = C^2 \left[\pi_i(\alpha_i A_i^2 + \delta_i D_i^2 + P_i^2) - \pi_i P_i^2\right], \quad (6)$$

where $\alpha$, $\delta$, $A$ and $D$ are the same functions of frequencies and $\beta$'s as defined previously for additivity between loci. When there is symmetry in effects, i.e., $\beta_{ij} = \beta_j$, and when the gene frequencies are the same at all loci, the total variance reduces to

$$\sigma_Y^2 = C^2 \left[(\alpha A^2+\delta D^2+P^2)^s - P^{2s}\right], \quad (7)$$

where each term in the expansion of $C^2(\alpha A^2+\delta D^2+P^2)^s$ corresponds to a type of variance except for $C^2P^{2s}$ which is the correction factor or square of the mean.

Some of the partitions for this model are given in Table 2 from which an extension to any type of partition should be apparent. The effect of inbreeding on the partitions varies and depends on gene frequencies and dominance. If there is no dominance in terms of the effects at a locus, i.e., $\beta_{i1} = (\beta_{i2}+\beta_{i0})/2$, all partitions involving dominance at this locus will disappear. If this were true of all loci the partitions would reduce to only those involving additive in their nomenclature.

The ratio of the dominance partition to the additive partition for each locus is the same function of the effects of the locus that is found in the model with additive effects between loci given just previously. A logarithmic transformation will reduce this model to the previous additive one as long as none of the $\beta$'s are zero or negative. However, by letting some of the $\beta$'s be zero the multiplicative description generates the complementary and duplicate factor models as special cases.

The complementary model is characterized by

$Y = C$ when all $j$'s are greater than 0
$= 0$ when any $j$ is 0,

TABLE 2

*Some partitions of variance for multiplicative action between loci*

| Number | Type | Variance | Total Variance |
|---|---|---|---|
| s | $a_i$ | $C^2\alpha_i A_i^2 \prod_{k\neq i}^{s} P_k^2$ | $\sigma^2_{10}$ |
| s | $d_i$ | $C^2\delta_i D_i^2 \prod_{k\neq i}^{s} P_k^2$ | $\sigma^2_{01}$ |
| s(s-1)/2 | $a_i x a_k$ | $C^2\alpha_i\alpha_k A_i^2 A_k^2 \prod_{\ell\neq i,k}^{s} P_\ell^2$ | $\sigma^2_{20}$ |
| s(s-1) | $a_i x d_k$ | $C^2\alpha_i\delta_k A_i^2 D_k^2 \prod_{\ell\neq i,k}^{s} P_\ell^2$ | $\sigma^2_{11}$ |
| s(s-1)/2 | $d_i x d_k$ | $C^2\delta_i\delta_k D_i^2 D_k^2 \prod_{\ell\neq i,k}^{s} P_\ell^2$ | $\sigma^2_{02}$ |
| s(s-1)(s-2)/6 | $a_i x a_k x a_\ell$ | $C^2\alpha_i\alpha_k\alpha_\ell A_i^2 A_k^2 A_\ell^2 \prod_{m\neq i,k,\ell}^{s} P_m^2$ | $\sigma^2_{30}$ |

and so on.

or in other words, an effect $C$ is found when no locus is homozygous recessive in contrast to what is found when one or more loci are homozygous recessive. This is a symmetrical model for which $\beta_{i2} = \beta_{i1} = 1$ and $\beta_{i0} = 0$ for all loci in the multiplicative model.

For the duplicate factor model

$Y = 0$ when any $j$ is greater than 0

$= C$ when all $j$'s are 0,

which says that the condition $C$ is found when all loci are homozygous recessive in contrast to the condition found when one or more loci are not homozygous recessive. This, of course, is another variant of the multiplicative model, $Y = C \prod_i \beta_{ij}$, when $\beta_{i2} = \beta_{i1} = 0$ and $\beta_{i0} = 1$ for all loci.

The partitions of variance for both the complementary and duplicate factor models can be found directly from those for the multiplicative model by making the appropriate substitutions.

*Optimum genetic model*

The optimum model, essentially the same as WRIGHT's (1935), and which readily reduces to the one of HORNER *et al.* (1955), is

$$Y = -(\sum_i \beta_{ij} - \theta)^2. \tag{8}$$

In this model the genes at a locus produce effects, $\beta_{ij}$'s, which are additive between loci (WRIGHT's primary scale), but which will vary in their effect on desirability, $Y$ (WRIGHT's secondary scale), according to the negative square of their deviation from some optimum value, $\theta$ (most desirable value). For two loci the model is

| | BB | Bb | bb |
|---|---|---|---|
| AA | $-(\beta_{12}+\beta_{22}-\theta)^2$ | $-(\beta_{12}+\beta_{21}-\theta)^2$ | $-(\beta_{12}+\beta_{20}-\theta)^2$ |
| Aa | $-(\beta_{11}+\beta_{22}-\theta)^2$ | $-(\beta_{11}+\beta_{21}-\theta)^2$ | $-(\beta_{11}+\beta_{20}-\theta)^2$ |
| aa | $-(\beta_{10}+\beta_{22}-\theta)^2$ | $-(\beta_{10}+\beta_{21}-\theta)^2$ | $-(\beta_{10}+\beta_{20}-\theta)^2$ |

(9)

All types of partitions of variance for the optimum genetic model are given in Table 3. For this model there are *only* two factor epistatic partitions, all higher factor partitions being zero.

The additive variance for a locus depends on, in addition to its own frequencies and effects, how much the sum of the averages of the effects on the primary scale deviates from the optimum. Likewise, the dominance variance depends on this deviation from the optimum and on the dominance of the effects for the primary scale. Even with no dominance of the effects for the primary scale there is still dominance variance for the secondary scale because of the term $(\beta_{i2} - \beta_{i0})^2/4$.

The epistatic partitions depend in no way on the value of the optimum but on the additive and dominance effects of the genes on the primary scale. When dominance is absent on the primary scale for all loci, only additive by additive epistatic partitions remain.

## DISCUSSION

This has been purposely a very general treatment of classical models. Specific values for any of the components of variance can be found by making numerical substitutions in the formulae. Similarly, numerical values for covariances of relatives can be found by substituting in the formulae for the covariances of relatives (COCKERHAM 1954). In this way one can indicate the bias in certain estimating procedures as did HORNER *et al.* (1955) and HORNER and KEMPTHORNE (1955) for special cases of these models. The problem that arises concerns the proper parameters of the model and the relationship between the genotypic values specified by the model and the phenotype.

The genotypic values, $Y$'s, specified by the model in the present paper have not

TABLE 3

*Partitions of variance for the optimum genetic model*

| Type | Variance |
|---|---|
| $a_i$ | $4\omega_i[(u_i+Fv_i)(\beta_{i2}-\beta_{i1})(\frac{\beta_{i2}+\beta_{i1}}{2}+\sum_{k\neq i}P_k-\theta)+(v_i+Fu_i)(\beta_{i1}-\beta_{i0})(\frac{\beta_{i1}+\beta_{i0}}{2}+\sum_{k\neq i}P_k-\theta)]^2.$ |
| $d_i$ | $4\delta_i[(\beta_{i2}-2\beta_{i1}+\beta_{i0})(\frac{\beta_{i2}+2\beta_{i1}+\beta_{i0}}{4}+\sum_{k\neq i}P_k-\theta)+\frac{(\beta_{i2}-\beta_{i0})^2}{4}]^2.$ |
| $a_i xa_k$ | $4\omega_i\omega_k A_i^2A_k^2$ |
| $a_i xd_k$ | $4\omega_i\delta_k A_i^2D_k^2$ |
| $d_i xd_k$ | $4\delta_i\delta_k D_i^2D_k^2$ |

been related to phenotypes. The $Y$'s may add or interact in some way with environment to form the phenotype. Also, the $Y$'s for any model may constitute only a portion of the genotypic value. For example, the genotypic value could be formed by simply adding the $Y$ values for the multiplicative and optimum models, each from independent sets of genes. In this case the components of variance would be a sum of those for the two models.

From the standpoint of distinguishing between models from data some restrictions or specifications of parameters must be made, or an exhaustive variation in the parameters must be considered. The feasibility of distinguishing between specific genetic models is questionable on three counts: (1) gene action for biometrical characters is probably not all of one type (2) variants of different models give similar results and (3) estimates from data generally have too large sampling errors to be very discriminatory. This does not mean that broad categories of models, such as additive, dominance and epistasis or combinations of these cannot be distinguished. It is feasible even, for example, that one could correctly conclude the epistasis to be mostly of the additive type or to be mostly two factor. However, in the latter case, the appropriate model may not be the optimum one since many genetic situations can give rise to only additive, dominance and two factor epistatic variance.

The duplicate factor and complementary models illustrate some of the variations that can be obtained from the multiplicative one. Even for the complementary and duplicate factor models the genotypic variance can be distributed among the partitions of variance in many ways and in duplicating ways by varying gene frequencies and the number of loci. However, considering only values greater than zero for the $\beta$'s they still can be varied to distribute the total variance

among the partitions in an almost unlimited number of ways. To reduce these variations to a manageable order HORNER *et al.* (1955) and HORNER and KEMPTHORNE (1955) fixed the total genotypic coefficient of variation to be 30 percent or less. The effect of this restriction will be illustrated.

In the symmetrical multiplicative model with gene frequencies the same at all loci, the total genotypic coefficient of variation, $V_y$, is related to the additive coefficient of variation, $V_a$, of a locus and the dominance coefficient of variation, $V_d$, of a locus as

$$1 + V_y^2 = (1+V_a^2+V_D^2)^s. \tag{10}$$

$$V_y = \sigma_Y/CP^\beta, \qquad V_a = \sqrt{A^2/P^2}, \qquad V_d = \sqrt{D^2/P^2}$$

The use of coefficients of variation removes from consideration variations in the constant $C$ or constant multiples of the $\beta$'s. The fraction of the total genotypic variance that is additive is a function of the coefficients of variation as

$$\sigma_{10}^2/\sigma_Y^2 = sV_a^2/V_y^2 \tag{11}$$

and similarly for the dominance variance,

$$\sigma_{01}^2/\sigma_Y^2 = sV_d^2/V_y^2 \tag{12}$$

Thus the sum of these two can be expressed entirely as a function of the numbe. of loci and the total genotypic coefficient of variation:

$$(\sigma_{10}^2+\sigma_{01}^2)/\sigma_Y^2 = s(V_a^2+V_d^2)/V_y^2 = s[(1+V_y^2)^{1/s}-1]/V_y^2 \tag{13}$$

This expression has the limit as $s$ becomes increasingly large of $[ln(1+V_y^2)]/V_y^2$, where $ln$ is the natural logarithm. Table 4 contains these fractions for selected values of $s$ and $V_y$.

TABLE 4

*Sum of the additive and dominance fractions of the total genotypic variance for selected values of* $V_y$ *and* s *in the multiplicative model*

| s | $V_y$ | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | .1 | .2 | .4 | .6 | .7 | .8 | .9 | 1 | 2 | 4 |
| 2 | 1.00 | 1.00 | .96 | .92 | .90 | .88 | .85 | .83 | .62 | .39 |
| 10 | 1.00 | 1.00 | .94 | .87 | .83 | .79 | .75 | .72 | .44 | .20 |
| ∞ | 1.00 | .98 | .93 | .85 | .81 | .77 | .73 | .69 | .40 | .18 |

The number of loci has little effect except for large values of $V_y$. Limiting the total genotypic coefficient of variation to 40 percent or less makes the multiplicative model an additive one without epistasis for all practical purposes, and the two could never be distinguished from data. Making $V_y$ small essentially makes the marginal effects small, in which case, it is generally known that the additive model is a good approximation to the multiplicative model. It is not necessary that the genotypic coefficient of variance be related to the phenotypic one, however, and with large $V_y$'s a considerable fraction of the total genotypic variance will be epistatic.

## SUMMARY

General expressions of the partitions (additive, dominance, additive × additive and so on) of variance were given for the additive, multiplicative, and optimum genetic models. Variants of the multiplicative model included the complementary and duplicate factor models as special cases. Some limitations in distinguishing between models from data were discussed. The effect of limiting the total genotypic coefficient of variation for the multiplicative model was investigated.

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# DIFFERENTIAL GENE RESPONSE TO MUTAGENS IN DROSOPHILA MELANOGASTER

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THE most outstanding recent development in the field of mutagenesis is the discovery that gene mutation is not random, but selective, depending on the nature of both mutagen and gene locus. The evidence for this selective mutagenicity became abundantly clear with the introduction of microorganisms (bacteria and the lower fungi) in the study of mutagenesis. The ease with which large populations of these organisms can be cultured, treated, and subsequently observed, helped in overcoming the technical difficulty arising from the rarity of the mutation event. DEMEREC (1954, 1955), working on *Escherichia coli*, could demonstrate that the rate of reversion (back mutation) at several nutritional deficiency loci varied markedly under similar treatments with different mutagens. Evidence was also available suggesting that this differential response was a function of the genes themselves, rather than the bacterial cell, since there were no particular strains which were conducive to high, or low, mutational rates for all loci. A grave drawback of the bacterial back mutation test, however, is that it is difficult (in some strains even impossible) to subject the arising mutants to recombination allelism tests. Most workers on bacterial mutagenesis, therefore, accept the scoring of the mutants on the basis of their phenotypic manifestations. This will inevitably lead to an erroneous assessment of the true mutation rate per locus since it will be impossible to eliminate cases due to adaptation or suppressor mutations. How far this error could have affected the phenomenon of differential mutability in bacteria is difficult to assess, but it is certain that it could not be the whole explanation. There are a number of mutagen stable loci, which do not respond at all to a diversity of strong mutagens highly effective on other genes.

The results with Neurospora are closely parallel to those with bacteria. Here again the rate of back mutation at different loci, or even at different alleles of the same locus, depends on the mutagen. This has been shown to occur with different radiations like ultraviolet and X-rays (GILES 1951), as well as with different chemical mutagens (KÖLMARK and WESTERGAARD 1953). Of particular interest are the results of KÖLMARK (1953) who demonstrated that two genes in the same nucleus could show different relative response to various mutagens. Unlike bacteria, the Neurospora technique enables the confirmation of the mutations induced, through adequate crosses and subsequent analysis of the segregation ratios among the ascospores. This makes the evidence for differential mutability based on the Neurospora test even more secure than that based on bacteria.

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aminophenylethylamine.

*Phenylcarboxylic mustards:*

$(ClCH_2CH_2)_2N.C_6H_4.(CH_2)_nCOOH$

CB.1331: n = 1 ; p-NN-di-(2-chloroethyl)aminophenylacetic acid.

CB.1332: n = 2 ; p-NN-di-(2-chloroethyl)aminophenylpropionic acid.

CB.1348: n = 3 ; p-NN-di-(2-chloroethyl)aminophenylbutyric acid, or "Chlorambucil".

CB.1356: n = 4 ; p-NN-di-(2-chloroethyl)aminophenylvaleric acid.

*Alkyl methanesulphonates:*

*Monofunctional* $R\text{-}OSO_2CH_3$

CB.1528: $R = C_2H_5$ ; ethyl methanesulphonate.

CB.1540: $R = CH_3$ ; methyl methanesulphonate.

*Difunctional* $CH_3O_2SO\text{-}R\text{-}OSO_2CH_3$

CB.2040: $R = (CH_2)_3$ ; 1:3-dimethanesulphonoxypropane.

CB.2041: $R = (CH_2)_4$ ; 1:4-dimethanesulphonoxybutane, or "Myleran".

CB.2058: $R = CH_2.C \equiv C.CH_2$ ; 1:4-dimethanesulphonoxybut-2-yne.

CB.2094 and CB.2095: $R = CH_2CH{=}CHCH_2$ ; the *cis*, and *trans* forms of 1:4-dimethanesulphonoxybut-2-ene.

CB.2511: $R = CH_2(CHOH)_4CH_2$ ; 1:6-dimethanesulphonyl mannitol.

*Halogeno-alkyl methanesulphonates* $X\ CH_2CH_2OSO_2CH_3$

CB.1506: X = Cl ; 2-chloroethyl methanesulphonate.

CB.1522: X = F ; 2-fluoroethyl methanesulphonate.

*Amino-acid mustards:*

*Monofunctional*

CB.1592: $ClCH_2CH_2SCH_2CH(NH_2)COOH$ ; S-2-chloroethylcysteine

*Difunctional*

CB.3007, CB.3025, CB.3026: $(ClCH_2CH_2)_2N.C_6H_4.CH_2CH(NH_2)COOH$ ; p-NN-di-(2-chloroethyl)aminophenylalanine, the DL mixture (Merphalan), the L-isomer (Melphalan), and the D-isomer (Medphalan).

CB.3051: $(ClCH_2CH_2)_2N.C_6H_4\text{-}O\text{-}C_6H_4.CH_2CH(NH_2)COOH$ ; p-/p′-NN-di-(2-chloroethyl)aminophenoxy/phenylalanine.

CB.1385: $(ClCH_2CH_2)_2N.C_6H_4.CH_2CH_2CH(NH_2)COOH$ ; α-amino-γ-p-(NN-di-2-chloroethyl amino)phenylbutyric acid.

For the mutagenicity tests the above compounds were administered as aqueous solutions by intra-abdominal injection round the testes of young imagines of Oregon-K Drosophila males. The carboxylic and amino acid mustards, which are practically insoluble in water, were converted to the sodium salts, and these were sufficiently soluble for the biological tests. Some of the di-methanesulphonates (e.g., CB.2041, 2058, 2094 and 2095) gave true solutions of a maximum concentration of about $0.8 \times 10^{-2}$ M. Whenever higher concentrations of these compounds were tested, they were administered as partial suspensions.

The radiation mutagenicity tests were carried out on males comparable to those used in the genetic experiments with the chemical mutagens. The males were of the same strain, as well as of nearly equal average weight and size. In all

experiments irradiation was done by the same X-ray machine: a "Picker" therapeutic machine, 250 kv-15 milliamps, with inherent filtration of 0.2 mm Cu and added filtration of 0.2 mm Sn + 0.25 mm Cu + 1 mm Al; the half value layer being 1.5 mm Cu. The flies were irradiated in batches of about 1,000 males in a perforated "perspex" cylindrical chamber (2.5 cms diameter × 1 cm height) placed 20 cms from the source. Under these conditions the dose rate delivered was 250–260r per minute. The exact dose rate for each irradiation was checked by a dosimeter, thus enabling an accurate determination of the total dose in each of the genetical experiments (Table 4).

*The detection of mutants:* The present analysis is confined to "visibles" induced in the X chromosome of the "Oregon-K" stock. The greatest majority of the mutants were detected by the Muller-5 technique, though a small number occurred in attached-X experiments and were also included. In Muller-5 tests, cultures yielding only M-5 males ($sc^{81}B(InS)w^{a}sc^{8}$/Y) and completely lacking in the potentially wild type male class, were scored as sex-linked recessive lethal cultures. The occurrence in $F_2$ cultures of non-Muller-5 males with anomalous phenotypes was taken as an indication of the induction of a sex-linked visible. The flies from these cultures carrying the treated chromosome were used to establish stocks, which were subsequently subjected to further genetic analysis. It thus became possible to determine whether the mutation was complete or mosaic in the germ line, its exact genetic position, and whether it was a recurrence at a known locus or a mutation of a hitherto unaffected gene.

Several control experiments were undertaken with untreated males to determine the spontaneous mutation rate using the above techniques. In 16 Muller-5 experiments there was a mean sex-linked recessive lethal rate of 0.18 ± 0.05 percent. As regards the visibles, there were two mutants (one rudimentary: *r*, and one unidentified body color mutant: a $\beta$ locus) in 13093 Muller-5 $F_2$ cultures, and also two others (one singed: *sn*, and one mosaic yellow: $y$) among 29663 $F_1$ males from attached-X experiments. The spontaneous $\beta$ locus mutant was not allelic to any of the induced mutations considered in the present analysis. The over-all control sex-linked recessive visible rate, therefore, for the X chromosome gene complement is 0.009 percent.

A study was also undertaken of the absolute mutation rate at certain specific X chromosome loci under the effect of X-rays and the amino acid mustard CB.3025 /p-NN-di-(2-chloroethyl) amino-*L*-phenylalanine/. The loci analysed in this way, together with their genetical positions are detailed in Tables 4 and 5, and their phenotypic description has already been published (FAHMY and FAHMY 1958b). The experimental procedure for both the physical and chemical agents consists of treating the wild type males (Oregon-K stock) and subsequently mating them to females either homozygous for several of the marked loci, or heterozygous for two sets of markers, each on one of the X chromosomes. When the normal allele of the marked locus in the paternal X has been affected, the $F_1$ daughter will reveal the mutation. The total number of the treated X chromosomes is given by the number of $F_1$ daughters observed when the mothers are

TABLE 1

*Distribution of alleles at specific α loci with different mutagenic agents*

| Locus | | Position | X-ray | TEM | Carboxylic mustards | Amino acid mustards | Sulphonates | C.B. 1506 | C.B. 1592 | C.B. 3034 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| yellow | *y* | 0.0 | 1 | 4 | 1 | 11 | 12 | 1 | 2 | 1 | 33 |
| silver | *svr* | 0.0 | 1 | 2 | 1 | 5 | 2 | .. | 2 | 1 | 14 |
| twisted | *tw* | 0.4 | 4 | 2 | 1 | 7 | 10 | 3 | 1 | 2 | 30 |
| broad | *br* | 0.6 | .. | .. | .. | .. | 1 | 1 | .. | .. | 2 |
| prune | *pn* | 0.8 | 1 | .. | .. | 4 | 6 | 1 | .. | .. | 12 |
| giant | *gt* | 0.9 | .. | .. | .. | .. | 2 | .. | .. | .. | 2 |
| white | *w* | 1.5 | 2 | 6 | 1 | 8 | 9 | 5 | 1 | .. | 32 |
| diminutive | *dm* | 4.6 | 3 | .. | .. | .. | .. | .. | .. | .. | 3 |
| echinus | *ec* | 5.5 | .. | .. | .. | 3 | .. | 1 | .. | .. | 4 |
| bifid | *bi* | 6.9 | .. | .. | .. | 1 | .. | .. | .. | 1 | 2 |
| ruby | *rb* | 7.5 | 3 | 1 | 1 | 4 | 2 | 2 | .. | 2 | 15 |
| rugose | *rg* | 11.0 | .. | 2 | .. | 2 | 4 | .. | .. | .. | 8 |
| roughex | *rux* | 15.0 | 1 | 1 | .. | 2 | .. | .. | .. | 1 | 5 |
| vesiculated | *vs* | 16.3 | 4 | 2 | .. | 9 | 6 | 3 | 1 | 1 | 26 |
| deltex | *dx* | 17.0 | .. | 1 | 1 | 2 | 2 | 1 | .. | .. | 7 |
| oval | *ov* | 17.5 | .. | .. | .. | 1 | 1 | .. | .. | .. | 2 |
| shifted | *shf* | 17.9 | 1 | 1 | .. | 1 | 8 | 2 | .. | .. | 13 |
| carmine | *cm* | 18.9 | .. | 2 | .. | 4 | 1 | 2 | .. | .. | 9 |
| scooped | *scp* | 19.3 | .. | .. | .. | .. | 1 | 1 | .. | .. | 2 |
| cut | *ct* | 20.0 | 2 | .. | 1 | .. | .. | .. | .. | .. | 3 |
| singed | *sn* | 21.0 | 2 | 4 | .. | 2 | 5 | 1 | 2 | .. | 16 |
| pentagon | *ptg* | 23.2 | .. | 1 | .. | .. | 2 | .. | .. | .. | 3 |
| goggle | *gg* | 25.0 | 1 | .. | 1 | 1 | 1 | .. | .. | .. | 4 |
| tan | *t* | 27.5 | 6 | .. | 1 | 4 | 3 | 1 | 1 | .. | 16 |
| lozenge | *lz* | 27.7 | 5 | 6 | .. | 6 | 1 | 3 | .. | .. | 21 |
| tarry | *ta* | 27.7 | 1 | .. | .. | 7 | 2 | .. | 1 | .. | 11 |
| divers | *dvr* | 28.1 | 1 | .. | .. | 2 | 2 | 1 | .. | .. | 6 |
| vermilion | *v* | 33.0 | 2 | 3 | .. | 6 | 9 | 2 | 2 | .. | 24 |
| miniature | *m* | 36.1 | 1 | 3 | 1 | 6 | 4 | 1 | 3 | .. | 19 |
| dusky | *dy* | 36.2 | 1 | 5 | .. | 3 | 8 | 1 | 3 | .. | 21 |
| furrowed | *fw* | 38.3 | 2 | 3 | .. | 3 | 4 | 3 | .. | .. | 15 |
| wavy | *wy* | 41.9 | .. | 3 | .. | 3 | 2 | .. | .. | .. | 8 |
| sable | *s* | 43.0 | .. | .. | .. | 1 | 2 | .. | .. | .. | 3 |
| garnet | *g* | 44.4 | 1 | 4 | .. | 5 | 7 | 1 | .. | .. | 18 |
| narrow abdomen | *na* | 45.2 | 3 | 2 | 2 | 3 | 5 | 3 | 2 | .. | 20 |
| pleated | *pl* | 47.9 | .. | 1 | .. | 4 | 1 | .. | .. | .. | 6 |
| scalloped | *sd* | 51.5 | .. | .. | .. | 2 | 1 | .. | .. | .. | 3 |
| uneven | *un* | 54.4 | .. | 1 | .. | 2 | 3 | .. | .. | .. | 6 |
| rudimentary | *r* | 54.5 | 5 | 11 | 2 | 14 | 25 | 5 | 2 | 4 | 68 |
| inflated | *if* | 55.0 | .. | 1 | .. | 1 | .. | .. | .. | .. | 2 |
| forked | *f* | 56.7 | 2 | .. | .. | 3 | 3 | 3 | .. | .. | 11 |
| fused | *fu* | 59.5 | 1 | 3 | .. | 6 | 11 | 1 | .. | .. | 22 |
| carnation | *car* | 62.5 | .. | .. | .. | 2 | .. | .. | .. | .. | 2 |
| Total | | | 57 | 75 | 14 | 150 | 168 | 49 | 23 | 13 | 549 |

of $\chi^2$. We therefore applied the $\Psi$ test (C. A. B. SMITH 1951, 1952) for the assessment of heterogeneity of proportions in a contingency table. This method is far less disturbed by small numbers in the individual cells than $\chi^2$. It is also to be noted that the tables under analysis have a large number of rows, a feature which favors the applicability of the $\Psi$ test, since it helps in smoothing out the irregularities due to the smallness of the individual numbers. The null hypothesis for this test is the absence of heterogeneity, which is to be interpreted as meaning that the distribution of alleles among the various X chromosome loci (under consideration in the contingency table) is independent of the mutagen.

Table 1 shows the distribution of alleles at 43 $\alpha$ loci for X-rays and seven classes of alkylating mutagens. The $\Psi$ test for this distribution corresponds to $P = 0.41$, which is clearly nonsignificant. The over-all relative mutability at the "classical" or $\alpha$ loci, therefore, does not vary with the mutagen. Table 2 gives the distribution of alleles at 63 $\beta$ loci, under the effect of X-rays, and five classes of the alkylating mutagens. In this analysis the mutants of TEM and the carboxylic mustards were omitted, since some of the experiments with these agents were undertaken before the accumulation of the $\beta$ loci. The $\Psi$ test for the data in Table 2 gave a $P = 0.01$, which is highly significant. Unlike the $\alpha$ loci, therefore, the relative response of the $\beta$ loci does differ under the various mutagens. Lastly, an over-all analysis has been undertaken of the relative mutability at 106 X chromosome loci ($43\text{-}\alpha + 63\text{-}\beta$) which have mutated more than once in our more recent experiments (i.e., with the mutagens detailed in Table 2). The $\Psi$ test for this over-all distribution gave $P = 0.02$, which is convincingly significant, indicating a differential response. It would be desirable to analyse the distribution of alleles at the various loci for pairs of mutagenic series, to determine the degree of differential action for the various agents. The samples for the individual series, however, are sufficiently large to warrant such analysis only in the case of the amino acid mustards and the sulphonates. A $\Psi$ test for allele distribution at all the $\alpha$ and $\beta$ loci under these two chemical series gave $P = 0.01$, indicating a real difference. This is of great interest, since it shows that different chemical series within the alkylating agents do produce differential mutagenic effects.

The most important outcome of the above statistical analysis is that there is a group of genes (the $\alpha$ loci) which seems to respond to roughly the same degree to radiation and the various alkylating mutagens. Another group of genes (the $\beta$ loci) do vary in their response according to the agent. To put this conclusion to further test, we pooled the number of alleles at the $\alpha$, as well as at the $\beta$ loci for each mutagen and compared their ratios in a $2 \times 6$ contingency table (Table 3). The numbers here are sufficiently high to justify the application of $\chi^2$ test. The total $\chi^2$ for the six mutagenic classes gave a value of 18.8 which for five degrees of freedom corresponds to $P = 0.004$. The relative effect of various mutagens on the $\alpha$ and $\beta$ loci is, therefore, most decisively different.

*Comparative mutation rates at specific loci:* To substantiate the discovery of the preferential response of certain genes to particular mutagens, tests have been undertaken to determine the exact mutation rates at specific $\alpha$ and $\beta$ loci under

TABLE 2

*Distribution of alleles at specific β loci with different mutagenic agents*

| Locus | | Stock | Position | X-ray | Amino acid mustards | Sulphonates | C.B. 1506 | C.B. 1592 | C.B. 3034 | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| scute$^{fahmy}$ | *sc$^{fah}$* | 2016 | 0.0 | .. | 2 | 1 | .. | .. | .. | 3 |
| tiddler | *tdd* | 2369 | 0.0 | .. | 1 | 1 | .. | .. | .. | 2 |
| unequal-wings | *uq* | 2197 | 0.5 | 1 | 2 | .. | .. | .. | .. | 3 |
| rough-eye-like | *re$^{2}$* | 1665 | 0.6 | .. | 2 | .. | .. | .. | .. | 2 |
| reduced-size | *rsi* | 1837 | 0.6 | .. | 2 | .. | 1 | .. | .. | 3 |
| murky | *mk* | 2064 | 0.8 | 1 | 4 | 3 | 2 | .. | 1 | 11 |
| faulty-chaete | *fc* | 2021 | 0.9 | .. | 1 | .. | .. | 1 | .. | 2 |
| waisted | *ws* | 2170 | 1.0 | .. | 1 | 1 | 1 | .. | .. | 3 |
| bent-scutellars | *bsc* | 2056 | 1.1 | .. | 3 | .. | .. | .. | .. | 3 |
| misproportioned | *mis* | 589 | 1.3 | .. | .. | 2 | .. | .. | 1 | 3 |
| vestigium | *ves* | 2134 | 1.4 | .. | 2 | .. | .. | .. | .. | 2 |
| cramped-like | *crm$^{2}$* | 2152 | 1.4 | .. | 4 | 3 | 2 | 1 | 1 | 11 |
| stiff-chaete | *sfc* | 2195 | 3.2 | .. | 1 | 1 | .. | 1 | .. | 3 |
| ruddle | *rud* | 1276 | 3.3 | 1 | 2 | 6 | .. | .. | .. | 9 |
| Ocellarless | *Oce* | 1243 | 5.7 | .. | 1 | 2 | .. | 1 | .. | 4 |
| microoculus | *mo* | 1998 | 6.7 | .. | 6 | 5 | 1 | 1 | .. | 13 |
| amber | *amb* | 2105 | 6.8 | .. | 2 | .. | 1 | .. | 2 | 5 |
| lacquered | *lac* | 1607 | 7.3 | 2 | 2 | 9 | 1 | .. | 2 | 16 |
| ommatoreductum | *omm* | 1255 | 12.8 | .. | 2 | 2 | .. | 1 | .. | 5 |
| deflected-wing | *dfw* | 2193 | 21.6 | 1 | 1 | .. | .. | .. | .. | 2 |
| slim-bristle | *smb* | 2368 | 23.1 | .. | . | 2 | .. | .. | .. | 2 |
| concave-wing | *ccw* | 1287 | 23.4 | .. | 3 | .. | .. | .. | .. | 3 |
| bright-eye | *bre* | 1415 | 24.6 | .. | 1 | 1 | .. | .. | .. | 2 |
| rearranged-tergites | *rea* | 1952 | 25.4 | .. | 2 | 1 | .. | 1 | .. | 4 |
| deltoid-veins | *dlv* | 2665 | 25.9 | .. | .. | .. | .. | 2 | .. | 2 |
| smaller | *sma* | 1726 | 29.9 | .. | 4 | 6 | .. | 1 | 1 | 12 |
| clumpy-marginals | *clm* | 1289 | 32.6 | .. | 2 | .. | 1 | .. | .. | 3 |
| wider-wing | *ww* | 1658 | 32.9 | .. | 2 | .. | .. | .. | .. | 2 |
| outshifted | *osh* | 2262 | 33.0 | 1 | .. | .. | 2 | .. | .. | 3 |
| costakink | *csk* | 1253 | 33.4 | 1 | .. | 1 | .. | .. | .. | 2 |
| slim | *slm* | 2183 | 33.7 | .. | 1 | .. | 3 | .. | .. | 4 |
| thread-bristle | *trb* | 2198 | 37.0 | .. | 2 | .. | .. | .. | .. | 2 |
| water-wings | *wtw* | 2013 | 38.9 | .. | 1 | 1 | .. | .. | .. | 2 |
| crumpled-tips | *crt* | 1227 | 40.3 | .. | 4 | .. | .. | 3 | 2 | 9 |
| upheld | *up* | 1892 | 41.0 | .. | 1 | 2 | .. | .. | .. | 3 |
| puny | *pun* | 1386 | 41.1 | .. | 1 | 1 | .. | .. | .. | 2 |
| tawny | *taw* | 2199 | 41.1 | .. | 2 | 1 | .. | .. | .. | 3 |
| cleft-end | *clf* | 1143 | 41.3 | .. | 2 | .. | .. | .. | .. | 2 |
| copper | *cop* | 1523 | 43.3 | .. | 2 | .. | .. | 1 | .. | 3 |
| rimy | *rim* | 2297 | 48.1 | .. | .. | 1 | 1 | 1 | .. | 3 |
| midget | *mgt* | 1879 | 49.6 | 2 | 4 | .. | 2 | .. | .. | 8 |
| thick-veins | *thv* | 2337 | 49.7 | .. | .. | .. | 1 | .. | 1 | 2 |
| slimma | *sla* | 1953 | 50.0 | .. | 6 | .. | .. | .. | .. | 6 |
| tiny-chaete | *tc* | 2161 | 51.6 | .. | 2 | .. | .. | .. | .. | 2 |
| minute-chaete | *mch* | 2416 | 52.0 | .. | 1 | 1 | .. | .. | .. | 2 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| droopy-wing | *drw* | 2889 | 52.3 | .. | 1 | .. | .. | 1 | .. | 2 |
| under-sized | *us* | X208 | 52.5 | 3 | .. | 1 | 1 | .. | .. | 5 |
| melanoscutellum | *ms* | 1916 | 52.6 | .. | 4 | 1 | 1 | .. | .. | 6 |
| Shaker-downheld | *Shw* | 2119 | 53.3 | 1 | 1 | 2 | .. | 2 | .. | 6 |
| small-wing | *sl* | 2100 | 53.5 | .. | 4 | 5 | .. | 1 | .. | 10 |
| acclinal-wing | *acc* | 2223 | 54.5 | .. | 2 | .. | .. | .. | .. | 2 |
| fine-lash | *fil* | 1464 | 56.8 | .. | 1 | 2 | .. | .. | .. | 3 |
| heldup | *hdp* | 2077 | 59.6 | 1 | 1 | .. | .. | .. | .. | 2 |
| tonochaete | *ton* | 1928 | 60.1 | .. | 1 | 1 | 1 | .. | .. | 3 |
| crooked-setae | *crk* | 1578 | 60.1 | .. | 2 | .. | .. | .. | .. | 2 |
| smalloid | *smd* | 2073 | 61.1 | 2 | 4 | 4 | 2 | 1 | 1 | 14 |
| megaoculus | *meg* | 2078 | 61.9 | .. | 2 | .. | .. | .. | .. | 2 |
| knobby-head | *kno* | 1223 | 63.9 | .. | .. | 2 | .. | .. | .. | 2 |
| melanised | *mel* | 1663 | 64.1 | .. | 2 | .. | .. | .. | .. | 2 |
| warty-like | *wa*$^2$ | 1392 | 64.4 | 1 | 2 | 2 | .. | .. | .. | 5 |
| bronzy | *bz* | 1920 | 64.9 | 1 | 3 | .. | .. | 1 | .. | 5 |
| refringent | *ref* | 2211 | 67.9 | .. | 2 | .. | .. | .. | 1 | 3 |
| little-fly | *lf* | 1981 | 68.3 | .. | 3 | 3 | 1 | 1 | .. | 8 |
| TOTAL | | | | 19 | 119 | 77 | 25 | 22 | 13 | 275 |

TABLE 3

*The ratio of alleles at the α and β loci, with different mutagenic agents*

| Mutagen | α loci mutants | β loci mutants | Total mutants | Ratio β/α loci mutants | $\chi^2$ |
|---|---|---|---|---|---|
| X-rays | 57 | 19 | 76 | 0.33 | 5.001 |
| Amino acid mustards | 150 | 119 | 269 | 0.79 | 5.350 |
| Sulphonates | 168 | 77 | 245 | 0.46 | 3.749 |
| CB. 1506 | 49 | 25 | 74 | 0.51 | 0.416 |
| CB. 1592 | 23 | 22 | 45 | 0.96 | 2.530 |
| CB. 3034 | 13 | 13 | 26 | 1.00 | 1.758 |
| TOTAL | 460 | 275 | 735 | 0.60 | 18.804 |

the effect of two contrasting mutagens. The two mutagens chosen were X-rays as a respresentative of the physical agents and the mustard derivative of phenylalanine [CB.3025, p-NN-di-(2-chloroethyl) aminophenylalanine] as a representative of the alkylating agents. This compound has been chosen for the test, since its mutagenic properties are appreciably different from those of radiation. It is a much weaker chromosome breaker, while being substantially more effective in the induction of point mutations resulting in recessive visibles (FAHMY and FAHMY 1956a).

An effort was made to standardize the experimental procedure in the genetic tests with the radiation and the chemical agents. The mutations were detected in the $F_1$ daughters from treated fathers mated to mothers carrying the marker loci. All the mutants were further analysed by the genetical tests detailed in the section on Technique. The range of mutagenic doses (as assessed by the lethal rate)

utilized with both mutagens was roughly the same. In all experiments only sperm delivered during the first nine days after treatment was tested, so as to ensure the homogeneity of the germ cell stages from which the mutants were recovered. Within this period, under our experimental conditions, only sperm and spermatids at the time of treatment are utilized. Another advantage of using the later sperm stages, is the avoidance of complications due to mutant "clusters" and possible germinal selection that may arise from the use of premeiotic stages.

Table 4, summarizes the data with 23 loci tested for mutability under the effect of X-radiation. Seven of these (insets in the table) are $\alpha$ loci, chosen from among the most common laboratory stocks, the remaining 16 are $\beta$ loci which were initially mutated by various alkylating mutagens. Two of the 16 loci here considered as $\beta$ (*viz.* small-wing: *sl*, and Turned-up-wing: *Tu*) have actually been recorded in the literature as occurring spontaneously or after irradiation. The marker alleles used in the present experiments, however, were chemically induced, involving the possibility that their substructure might be different. This could affect the detection of mutations to the specific alleles used. These loci, therefore, were included among the chemically responsive $\beta$ class. The chosen $\beta$ loci were distributed along the whole length of the X chromosome and were

TABLE 4

*Mutability at specific loci under X-radiation*

| Locus | | Stock | Position | Chromosomes chr. | Dose r | chr. × r ×10⁻⁸ | Deficiencies No. | Deficiencies Rates ×10⁻⁸ | Visibles No. | Visibles Rates ×10⁻⁸ |
|---|---|---|---|---|---|---|---|---|---|---|
| brachymacrochaete | *brc* | 1230 | 0.0 | 28626 | 3834 | 1.10 | .. | ... | .. | ... |
| scute | *sc* | .... | 0.0 | 38352 | 2990 | 1.15 | 2 | 1.74 | 4 | 3.49 |
| notchoid | *nd* | 638 | 2.7 | 34664 | 4266 | 1.48 | .. | ... | .. | ... |
| ruddle | *rud* | 1276 | 3.3 | 47272 | 3418 | 1.62 | 2 | 1.24 | .. | ... |
| Ocellarless | *Oce* | 1243 | 5.7 | 28626 | 3834 | 1.10 | 2 | 1.82 | .. | ... |
| microoculus | *mo* | 1998 | 6.7 | 58833 | 2678 | 1.58 | 2 | 1.27 | .. | ... |
| ommatoreductum | *omm* | 1255 | 12.8 | 28626 | 3834 | 1.10 | 2 | 1.82 | .. | ... |
| cut | *ct* | .... | 20.0 | 38352 | 2990 | 1.15 | 6 | 5.23 | 1 | 0.87 |
| concave-wing | *ccw* | 1287 | 23.4 | 44071 | 2990 | 1.32 | 2 | 1.52 | .. | ... |
| smaller | *sma* | 1726 | 29.9 | 47272 | 3418 | 1.62 | .. | ... | .. | ... |
| vermilion | *v* | .... | 33.0 | 89324 | 3207 | 2.86 | 2 | 0.70 | 4 | 1.44 |
| upheld | *up* | 1892 | 41.0 | 61422 | 2777 | 1.71 | 2 | 1.17 | .. | ... |
| wavy | *wy* | .... | 41.9 | 38352 | 2990 | 1.15 | 7 | 6.10 | 4 | 3.49 |
| copper | *cop* | 1523 | 43.3 | 28626 | 3834 | 1.10 | .. | ... | .. | ... |
| garnet | *g* | .... | 44.4 | 38352 | 2990 | 1.15 | 6 | 5.23 | 5 | 4.36 |
| tiny-chaete | *tc* | 2161 | 51.6 | 44071 | 2990 | 1.32 | .. | ... | .. | ... |
| small-wing | *sl* | 2100 | 53.5 | 44071 | 2990 | 1.32 | .. | ... | .. | ... |
| forked | *f* | .... | 56.7 | 38352 | 2990 | 1.15 | 1 | 0.87 | 3 | 2.62 |
| Turned-up-wing | *Tu* | 2212 | 59.1 | 47272 | 3418 | 1.62 | .. | ... | .. | ... |
| smalloid | *smd* | 2073 | 61.1 | 22278 | 4266 | 0.95 | 1 | 1.05 | 8 | 8.42 |
| carnation | *car* | .... | 62.5 | 66978 | 3418 | 2.29 | 7 | 3.06 | .. | ... |
| melanised | *mel* | 1663 | 64.1 | 32867 | 3316 | 1.09 | .. | ... | .. | ... |
| bronzy | *bz* | 1920 | 64.9 | 44071 | 2990 | 1.32 | 8 | 6.07 | .. | ... |

fully penetrant as well as fully viable and fertile in the homozygous condition. The phenotypic expression of all mutants at these loci is sufficiently clear to enable ease of detection and classification.

As far as the intragenic changes (point mutations: fully viable visibles) are concerned, it is abundantly clear that the $\beta$ loci are comparatively more resistant to radiation than the $\alpha$ loci. Only one of the 16 $\beta$ loci, *viz.* smalloid, was mutated by X-rays, and at an exceptionally high rate ($8.42 \times 10^{-8}$ per r). This is obviously a radiation responsive locus which has been missed by earlier workers. A consideration of the intergenic mutations (deficiencies and deletions covering the markers) conveys a certain degree of differential effect, though to a lesser extent than for the intragenic changes. On the whole the frequency of deficiencies at the $\alpha$ loci is higher than at the $\beta$ loci; eight of the 23 loci were refractory to radiation breakage (i.e., not involved in deficiencies) and all of these were among the $\beta$ loci. This is so, in spite of the fact that the sample-dose product for most of the noneliminated loci is higher than that for the responsive ones, and with a larger dose contribution. Their lack of response, therefore, cannot be entirely due to inadequate sample size, but is an indication that chromosome breakage, even under radiation, is not as random as is generally assumed.

Of the 23 loci tested under radiation, 11 were selected for testing under the effect of the mustard derivative of phenylalanine: ten of the $\beta$ loci (nine X-ray refractory and smalloid) and vermilion of the $\alpha$ loci. The results are detailed in Table 5. The chromosome sample tested per locus is reasonably within the range of that used in the radiation tests. The injected dose of the mustard has been carefully gauged to induce 6-8 percent sex-linked recessive lethals, so as to make the mutagenic doses with the chemical agent approximately equivalent to those in the radiation experiments. The actual mutagenic dose in each experiment was accurately determined by a sex-linked recessive lethal test on an aliquot of the

TABLE 5

*Mutability at specific loci under the "mustard" derivative of phenylalanine (L-p-NN-di(2-chloroethyl) aminophenylalanine)*

| Locus | | Stock | Position | Chromosomes chr. | Dose r equiv. | chr. × r equiv. ×10⁻⁸ | Deficiencies No. | Deficiencies Rates ×10⁻⁸ | Visibles No. | Visibles Rates ×10⁻⁸ |
|---|---|---|---|---|---|---|---|---|---|---|
| brachymacrochaete | *brc* | 1230 | 0.0 | 67445 | 3152 | 2.13 | .. | ... | 1 | 0.47 |
| notchoid | *nd* | 638 | 2.7 | 67445 | 3152 | 2.13 | 12 | 5.63 | 1 | 0.47 |
| ruddle | *rud* | 1276 | 3.3 | 44051 | 3465 | 1.53 | .. | ... | 4 | 2.61 |
| Ocellarless | *Oce* | 1243 | 5.7 | 67445 | 3152 | 2.13 | 1 | 0.47 | 1 | 0.47 |
| concave-wing | *ccw* | 1287 | 23.4 | 87547 | 2563 | 2.24 | .. | ... | 1 | 0.45 |
| vermilion | *v* | .... | 33.0 | 129831 | 2563 | 3.33 | 2 | 0.60 | 3 | 0.90 |
| copper | *cop* | 1523 | 43.3 | 67445 | 3152 | 2.13 | 2 | 0.94 | 1 | 0.47 |
| tiny-chaete | *tc* | 2161 | 51.6 | 48977 | 2563 | 1.26 | .. | ... | 1 | 0.79 |
| small-wing | *sl* | 2100 | 53.5 | 74253 | 2563 | 1.90 | .. | ... | 6 | 3.16 |
| smalloid | *smd* | 2073 | 61.1 | 67445 | 3152 | 2.13 | 1 | 0.47 | 8 | 3.76 |
| bronzy | *bz* | 1920 | 64.9 | 47036 | 2563 | 1.21 | 1 | 0.83 | 2 | 1.65 |

$u$, is a random variable which, according to the hypothesis under test, has a zero mean and unit variance. The significance of $u$, therefore, can be tested by referring its value to a table of the normal probability integral.

The intragenic mutation rates are compared in Table 7. The deviation from equal mutability reached decisive statistical significance ($P = 0.02$) for two loci —ruddle and small-wing, both of which were more responsive to the chemical agent. For two more loci, smalloid and bronzy, the deviation from equality reached the verge of significance; smalloid being more responsive to X-rays, and bronzy more so to the mustard. The over-all mutation incidence for the 11 loci does indicate a difference in the average mutation rate, but only on the border line of significance. This, however, is fortuitous, and is entirely due to the exceptionally high response of the smalloid locus to X-rays. When this locus is excluded, the difference in the mutability at the remaining ten loci proves to be highly significant ($P = 0.004$). A comparison was also undertaken for the mutability at the nine $\beta$ loci which proved refractory to X-radiation, taking into consideration the stronger treatment with the chemical agent (as measured by the chromosome-r product). This comparison revealed a very gross deviation from equal mutability under the two mutagens ($P = 0.0002$).

It would be interesting to determine whether differential gene response to mutagens is quantitative: that is a matter of degree, or qualitative: that particular loci are responsive to certain mutagens and completely refractory to others. The

TABLE 7

*Comparison of the mutation rates of the same gene locus under X-radiation and the "mustard" derivative of phenylalanine*

| Locus | | Mustard | | X-rays | | Pearson's ratio | |
|---|---|---|---|---|---|---|---|
| | | No. $a_1$ | Sample chr. $\times r(\times 10^{-8})$ $n_1$ | No. $a_2$ | Sample chr. $\times r(\times 10^{-8})$ $n_2$ | $u$ | P |
| *brc* | 1230 | 1 | 2.13 | 0 | 1.10 | 0.72 | 0.24 |
| *nd* | 638 | 1 | 2.13 | 0 | 1.48 | 0.83 | 0.20 |
| *rud* | 1276 | 4 | 1.53 | 0 | 1.62 | 2.06 | 0.02 |
| *Oce* | 1243 | 1 | 2.12 | 0 | 1.10 | 0.72 | 0.24 |
| *ccw* | 1287 | 1 | 2.24 | 0 | 1.32 | 0.77 | 0.22 |
| *v* | .... | 3 | 3.33 | 4 | 2.86 | 0.58 | 0.28 |
| *cop* | 1523 | 1 | 2.13 | 0 | 1.10 | 0.72 | 0.24 |
| *tc* | 2161 | 1 | 1.26 | 0 | 1.32 | 1.02 | 0.15 |
| *sl* | 2100 | 6 | 1.90 | 0 | 1.32 | 2.04 | 0.02 |
| *smd* | 2073 | 8 | 2.13 | 8 | 0.95 | 1.66 | 0.05 |
| *bz* | 1920 | 2 | 1.21 | 0 | 1.32 | 1.48 | 0.07 |
| Total | 11 loci | 29 | 22.12 | 12 | 15.49 | 1.55 | 0.06 |
| *smd* excluded | 10 loci | 21 | 19.99 | 4 | 14.54 | 2.65 | 0.004 |
| X-ray refractory | 9 loci | 18 | 16.66 | 0 | 11.68 | 3.55 | 0.0002 |

Pearson's ratio.

$$u = \frac{a_1 - r\lambda}{\sqrt{[r\lambda(1-\lambda)]}} \qquad \text{where } r = a_1 + a_2 \qquad \lambda = \frac{n_1 k}{n_1 k + n_2}; \qquad k = 1 \text{ for true equality.}$$

magnitude of the present data, does not permit a decisive answer to this problem. It is clear, however, that at least for ruddle and bronzy, the differential response to the amino acid mustard as compared to X-rays is only quantitative. Although these loci gave significantly lower mutation rates under X-rays (Table 7), they obviously can be mutated by it, since they were detected in our Muller-5 radiation tests (Table 2).

## DISCUSSION

The genome of Drosophila was shown to consist of two classes of genes, which differ in their stability and manner of response to mutagens. On the X chromosome, for example, there are a number of loci, here designated as the $\alpha$ class, which seem to respond to radiation, as well as to a diversity of chemical mutagens belonging to the so-called biological alkylating agents (chloroethylamines, ethyleneimines, and methanesulphonic esters). Morphologically detectable mutants, or 'visibles', at these loci have frequently been identified after the discovery of the mutagenic action of radiation, and a large sample of them has already been analysed and described in the Drosophila mutants literature (mainly BRIDGES and BREHME 1944). These 'visibles' came to be maintained in the various Drosophila laboratories and are looked upon as the 'classical' examples of morphological mutants. More recently, it was noticed (FAHMY and FAHMY 1956a, 1957a) that in our experiments with the alkylating mutagens, a great many mutants occurred in addition to the classical ones. As is demonstrated in this communication, the additional mutants are mainly the result of the response of another group of loci which have been designated the $\beta$ loci to distinguish them from the 'classical', or $\alpha$ loci.

Major differences have already been recognized between the $\alpha$ and $\beta$ classes of loci, as regards

(a) phenotypic manifestations
(b) distribution along the chromosome
(c) response to different mutagens.

Visible mutants at the $\beta$ loci are on the whole markedly more pleiotropic in their phenotypic manifestations, affecting several parts of the fly's body simultaneously. Many of these mutants are slow in development and, therefore, late in eclosion and also have reduced viability and/or fertility. The distribution of the $\beta$ loci recessive 'visibles' along the genetical map of the X chromosome has been compared with the corresponding $\alpha$ loci mutants (FAHMY and FAHMY 1957a, 1958a) and there was clear evidence for a higher relative frequency of the $\beta$ loci visibles at the proximal half of the chromosome, near the centromere. Whereas most of the $\alpha$ loci seem to respond to roughly the same degree under radiation and a diversity of chemical mutagens, the great majority of the $\beta$ loci do respond differentially. Another interesting difference is that the $\beta$ loci are on the whole less responsive to mutagens than the $\alpha$ class. This is clearly manifested in the frequency of allele recurrence under the same mutagens. The mean number of alleles per locus for the $\alpha$ class is $1.78 \pm 0.18$ (based on Table 1, excluding

TEM and the carboxylic mustards) while the comparable value for the $\beta$ loci for the same sample of treated chromosomes and under the same agents is $0.73 \pm 0.06$ (based on Table 2), which is clearly very significantly lower ($P = 10^{-8}$).

It cannot be overemphasized, however, that the classification of the gene loci into $\alpha$ and $\beta$ categories as given in the present communication, is by necessity neither rigid nor final. Any permanent grouping in this respect, is only possible after the accumulation of considerable data on the mutability of each locus to various mutagens. Whether this seemingly prohibitive task can ever be achieved, still remains to be seen. The present data, however, have clearly shown the reality of such a classification, even though it is virtually certain that the details of the grouping will change for some loci.

The differentiation of the gene complement into loci of different stability to mutagens is of great evolutionary significance. Mutation by permitting inherited variability, is perhaps the most important positive factor in the evolutionary process. On the other hand, it is easy to see that equal mutation at both the "advantageous" and "disadvantageous" loci would certainly have a hindering effect on evolution. There will be no chance for natural selection to build up the "desirable" genic combinations. In view of the fact that the majority of the $\beta$ loci "visibles" also have adverse effects on viability and fertility, it is perhaps not surprising that they were "stabilized" in the course of evolution. Of great interest in this connection, is the fact that practically none of the $\beta$ loci "visibles" were reported as having occurred spontaneously. While it is certain that this does not mean that none of them could have arisen in nature, it does, nevertheless, indicate that their spontaneous incidence is extremely rare, much rarer than it is for the $\alpha$ loci.

The mechanism of gene stabilization to mutagens can, at present, be only a subject for speculation. It is generally agreed, however, that gene stabilization could have occurred in evolution through the process of duplication, and it is possible that the same process may have played a rôle in the "protection" of some $\beta$ loci against mutagens. That duplications did occur in evolution, is indicated by the presence of *repeats* in the salivary chromosomes of various Diptera, including Drosophila. These are segments with similar or identical banding patterns, and which occasionally pair with one another in the same salivary nucleus. The so-called doublets (two identical bands) are generally believed to represent a single locus and they are probably the physical manifestation of duplication at the gene level. In a newly arisen gene duplication, the locus involved will be represented four times in a diploid organism. Physiologically, two of these are in effect superfluous and could probably be mutated or even eliminated without adverse effect on the organism. The evolutionary advantage of this in relation to grossly harmful mutations is obvious. Mutations which would be lethal, or which would at least lower the viability or fertility of the organism if they occurred in a nonduplicated locus, would probably have less drastic effects if occurring in a quadruplicated site.

The same situation, of course, holds in the case of a recessive "visible." Both halves of the duplicated locus will have to be mutated simultaneously, or consecutively, before the mutation is expressed phenotypically, even in the hemizygous condition. This will naturally result in a much lower mutation incidence at the duplicated as compared to the single loci. In gene duplicates of greater antiquity, it is highly probable that the two halves underwent some variation by independent mutation. This could be visualized to lead either to two independent loci, or alternatively to one integrated locus, with the various physical entities differentiated to undertake the various biochemical processes necessary for the physiological expression of the gene. From the mutagenesis point of view, loci which are duplications by origin, will be expected to be less mutable, since more than one physical entity of the locus may need to be damaged before the change is manifested. They will also be more likely to respond to agents with less localized "hits." At present, there seems to be no reliable means to estimate the area of action of a single "hit" for the various mutagens. It was shown, however, that recessive lethals induced by some of the alkylating mutagens are more frequently associated with cytologically detectable small deficiencies than the comparable X-ray mutations (BIRD and FAHMY 1953; FAHMY and BIRD 1953; FAHMY and FAHMY 1956a). This may be an indication of more "diffuse hits" by the chemical agents, which would favor simultaneous action on several genic subunits. On this basis, the relatively higher response of $\beta$ loci to the phenylalanine mustard as compared to X-rays, may well be an indication that these loci are physically more complex, probably through being gene duplications by origin.

The differential response to mutagens of the various loci especially within the $\beta$ class, is the most interesting recent development in mutagenesis studies. While its "exact" mechanism is still obscure, some recent advances in genetics and biochemistry may help in its elucidation. Genetical analysis of the highest resolution (BRENNER *et al.* 1958), for example, has revealed the high complexity of gene structure and how its subunits could be affected differently by various mutagens. The *r*II region of bacteriophage $T_4$ was shown to consist of two "cistrons" each of which is composed of a large number of mutable elements of several kinds. The mutational sites in each cistron which responded to 5-bromouracil, were different from those that reacted to proflavin. It is, therefore, clear that an individual locus could be of sufficient organizational complexity to allow for differential response to mutagens. To this there is to be added the variation in organization of different genes along the chromosomes of Metazoa which must have occurred during speciation and evolution. This complexity of organization within genes, as well as between them, implies a multiplicity of "receptors and reception mechanisms" to mutagens which may well result in some degree of differential locus response.

It would be indeed fascinating to identify the various mutagen "receptors" in the genetic material and determine how they interact with the various "active" groups of mutagens. This has not yet been achieved, but there is already some chemical evidence suggesting differences in the mode of action of the alkylating

SUMMARY

The gene loci on the X chromosome yielding recessive "visibles" could be classified into two classes on the basis of their response to mutagens. The $\alpha$ class respond to roughly the same degree to radiation and various alkylating mutagens. The $\beta$ class are on the average less responsive to the same mutagens than the $\alpha$ class. The $\beta$ loci also show a marked differential response to various mutagens which is manifested in: a) the proportion of alleles at the various loci recovered from the same sample of treated chromosomes, b) the ratio of mutants at the $\alpha$ to $\beta$ loci, and c) the absolute mutation rate at specific $\beta$ loci under comparable treatments with different mutagens (X-rays and the mustard derivative of phenylalanine). The possible reasons for the observed differential mutability and the evolutionary implications of the phenomenon are discussed.

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# GENETIC FACTORS AFFECTING MATING TYPE FREQUENCIES IN VARIETY 1 OF TETRAHYMENA PYRIFORMIS[1]

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PREVIOUS studies on mating type determination in variety 1 of *Tetrahymena pyriformis* have shown that cells with identical genotypes may manifest different mating types, and that the "hereditary" expression of mating type depends upon a persistent nuclear alteration of an unknown kind (NANNEY and CAUGHEY 1953; NANNEY 1956, 1958). Other studies have shown that the differences in the array of mating types that can be expressed by two homozygous inbred strains derived from a common source are determined by differences at a single chromosomal locus, designated as the *mt* locus (NANNEY, CAUGHEY and TEFANKJIAN 1955). Strains of "Family A" are homozygous for an allele which permits the development of clones (caryonides) of mating types I, II, III, V and VI in characteristic frequencies; strains of "Family B" are homozygous for another allele and produce caryonides of types II, III, IV, V, VI or VII.

Since these two alleles were obtained from the progeny of only two cells, isolated from the same pond, the existence of many alleles in natural populations seemed a likely possibility. Since such alleles might prove of considerable value in the analysis of the "epigenetic" system regulating mating type expression, a search was begun for new mating type alleles in other strains. Some evidence for relevant genic diversity has been obtained, but these genes do not determine new mating types or new combinations of the previously known mating types; they merely affect the frequencies with which the same combinations of mating types arise among the caryonides of a strain.

## MATERIALS AND METHODS

The number of strains of variety 1 from diverse natural sources is not large. WH-6 (I) and WH-14 (II) were collected from the same pond near Woods Hole, Massachusetts (ELLIOTT and GRUCHY 1952). WH-52 (III) was collected from a nearby pond at the same time (ELLIOTT and HAYES 1953). From extensive collections over much of the United States GRUCHY (1955) identified an additional variety 1 strain only from Vermont; UM-226 (VI) was chosen to represent this source. In the spring of 1955 the author made collections in Michigan and

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obtained variety 1 from Lake Huron near Alpena, Michigan, and from Indian Lake in Michigan's Upper Peninsula; strains Alp-4 (VI) and I.L.-12 (III) were chosen to represent these sources.

The procedures used in maintaining, crossing and testing cultures for mating types have been described previously (NANNEY, CAUGHEY and TEFANKJIAN 1955; NANNEY 1956) and will not be presented here.

## RESULTS

### *The mating type potentialities of strains of diverse origin*

The first objective of this study was to define as precisely as possible the capabilities of the various strains, in terms of the mating types which they could produce. One indication of capacity is the mating type expressed by the strain; thus WH-6 expresses type I; WH-14 expresses type II; WH-52 and I.L.-12 express type III; Alp-4 and UM-226 express type VI. Since earlier work had shown that a strain can express only one of its several potentialities, the phenotype of a clone provides only limited information concerning its capacities. Only breeding performance can provide information on the full range of potentialities.

The most direct breeding analysis would consist of crossing a single wild strain with itself, but this procedure is not possible in the absence of autogamy or selfing. Secondly, one might initiate a breeding analysis by crossing two clones of different mating type derived from the same source; but only in the case of WH-6 and WH-14 have diverse mating types been isolated from the same container. Hence, the only procedure available was to cross the wild strains to inbred strains of known capabilities. This procedure permits some additional information to be obtained, but does not allow a definition of the full range of potentialities. Thus, Family A cannot produce types IV or VII; the appearance of either of these types among the progeny of crosses between a wild strain and Family A would indicate that the wild strain carried the potentiality for the type. Similarly, if a new type appeared under these conditions its determinant must have come from the wild strain. Identical considerations apply to crosses with Family B. If type I or a new type appears in a cross to Family B, the determinant must have come from the wild strain. A combination of crosses with Families A and B yields information about the ability to produce types I, IV, VII and new types, but provides no evidence concerning capacities to produce types II, III, V or VI.

The analysis involved examination of both $F_1$ and $F_2$ generations. Earlier studies on variety 1 had shown that the ability to produce a mating type behaved as a dominant (or semidominant) trait, i.e., the $F_1$ produced in crosses of homozygous strains included some caryonides of each mating type possible in either parental line when sufficient clones were examined. If this observation could be generalized, the definition of mating type arrays in wild strains could be accomplished by ascertaining the mating types of the $F_1$ progeny. If, however, the ability to express some mating type should depend on a recessive gene, the $F_1$ strains would have to be crossed to yield an $F_2$. New genotypes might be found among the $F_2$ which might potentiate new mating types. In no case, however,

was a different array of types found in the $F_1$ and $F_2$ when adequate samples of caryonides were examined; for this reason the two sets of data are not separated in Table 1.

Among the $F_1$ and $F_2$ caryonides examined no new mating types appeared. Either the wild strains possess no new potentialities or they express them too infrequently to be detected under the conditions employed. Some of the samples are small, primarily because of difficulties with viability, but further studies with the derived strains have also failed to yield new types. Potentialities for new types in these strains must, therefore, have a very low probability of expression if they occur at all.

Table 1 also suggests that of the six strains examined only WH-14 has the ability to produce types IV or VII, and that all strains can produce mating type I. Since WH-6 and WH-14 were the progenitors of Families A and B, and since only WH-14 can produce types IV and VII, WH-14 must have been the source of the Family B *mt* allele. WH-14 also produces mating type I, however, a capacity not associated with the Family B allele. WH-14 is, therefore, probably heterozygous, carrying one Family B *mt* allele and one similar to or identical with the Family A allele. WH-6 may be homozygous for the Family A allele.

The other four strains lack the Family B allele, i.e., they cannot produce types IV and VII. They may have alleles similar to the Family A allele, but this possibility requires further exploration.

TABLE 1

*Screening tests for mating type potentialities by crosses of wild strains to inbred strains of known capabilities*

A. Crosses to Family A

| Strain | Mating type | Generation | Mating types of progeny | | | | |
|---|---|---|---|---|---|---|---|
| | | | Family A types | IV | VII | New | Total |
| WH-6 | I | $F_1$ | 26 | 0 | 0 | 0 | 26 |
| WH-14 | II | $F_1$, $F_2$ | 28 | 9 | 1 | 0 | 38 |
| WH-52 | III | $F_1$ | 45 | 0 | 0 | 0 | 45 |
| UM-226 | VI | $F_1$, $F_2$ | 13 | 0 | 0 | 0 | 13 |
| Alp-4 | VI | $F_1$, $F_2$ | 73 | 0 | 0 | 0 | 73 |
| I.L.-12 | III | $F_1$ | 85 | 0 | 0 | 0 | 85 |

B. Crosses to Family B

| Strain | Mating type | Generation | Mating types of progeny | | | |
|---|---|---|---|---|---|---|
| | | | Family B types | I | New | Total |
| WH-6 | I | ($P_1$) | .. | (1) | . | (1) |
| WH-14 | II | $F_1$ | 14 | 3 | 0 | 17 |
| WH-52 | III | $F_1$, $F_2$ | 77 | 6 | 0 | 80 |
| UM-226 | VI | $F_1$, $F_2$ | 88 | 17 | 0 | 105 |
| Alp-4 | VI | $F_1$, $F_2$ | 108 | 19 | 0 | 127 |
| I.L.-12 | III | $F_1$, $F_2$ | 90 | 19 | 0 | 109 |

*The extraction of mating type determinants from wild strains*

In the absence of better "indicator strains" the full array of mating type potentialities of a strain cannot be directly assessed. Procedures are available, however, for extracting genes from such strains and establishing them in homozygous condition. A wild strain capable of producing type I can be crossed to Family B. The $F_1$ progeny should contain a *mt* allele from Family B and also a *mt* allele from the wild strain. One quarter of the $F_2$ should lack the Family B allele entirely and should be homozygous for the corresponding gene in the wild strain. These homozygous segregants can be identified in progeny tests by their failure to produce types IV and VII. Their identification is facilitated by selecting $F_2$ segregants which lack types IV and VII among the four caryonides of a pair. This extraction procedure has been carried out for *mt* alleles from Alp-4 and UM-226. The performance of the derived strains is summarized in Tables 2 and 3.

The UM-226 derivative, identified by its failure to produce types IV and VII in the $F_3$, failed to produce these types in further inbreeding. One may be confident, therefore, that the Family B allele had been replaced. These strains continued to produce types I, II, III, V and VI, the same array of types found in Family A. The different progenies show significant variations in mating type frequencies, suggestive of further segregation of factors related to mating type

TABLE 2

*Mating type frequencies among caryonides produced in crosses of clones homozygous for the UM-226* mt *allele*

| Parental mating type | Generation | Mating types of progeny | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | VI | VII | |
| II × VI | $F_3$ | 15 | 9 | 1 | 0 | 5 | 9 | 0 | 39 |
| I × III | $F_4$ | 4 | 9 | 3 | 0 | 0 | 19 | 0 | 34 |
| I × III | $F_5$ | 8 | 12 | 4 | 0 | 2 | 23 | 0 | 49 |
| V × VI | $F_6$ | 12 | 19 | 2 | 0 | 3 | 21 | 0 | 58 |
| I × II | $F_7$ | 7 | 30 | 56 | 0 | 8 | 31 | 0 | 132 |

TABLE 3

*Mating type frequencies among caryonides produced in crosses of clones homozygous for the Alp-4* mt *allele*

| Parental mating type | Generation | Mating types of progeny | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | VI | VII | |
| I × VI | $F_3$ | 37 | 11 | 11 | 0 | 0 | 13 | 0 | 72 |
| I × II | $F_3$ | 35 | 7 | 5 | 0 | 0 | 17 | 0 | 64 |
| I × III | $F_4$ | 29 | 4 | 4 | 0 | 1 | 16 | 0 | 54 |
| I × II | $F_4$ | 12 | 1 | 3 | 0 | 0 | 3 | 0 | 19 |
| I × VI | $F_4$ | 41 | 6 | 2 | 0 | 0 | 5 | 0 | 54 |
| II × VI | $F_4$ | 36 | 7 | 2 | 0 | 0 | 6 | 0 | 51 |
| I × II | $F_5$ | 22 | 6 | 4 | 0 | 0 | 10 | 0 | 42 |

expression, but the array of types appears to be fixed at the time homozygosis is established at the *mt* locus. Whether the *mt* allele from UM-226 is identical with that in Family A cannot be determined from the available information.

The derivatives from Alp-4 behaved much like those from UM-226. Types IV and VII did not reappear, and types I, II, III and VI were produced regularly. However, mating type V appeared in neither of the progeny tests ($F_3$) on segregants and appeared only once in a subsequent generation. The mating type frequencies in the Alp-4 lineages were less erratic than those observed in the UM-226 derivatives. Again the data are suggestive of an allele similar to the Family A allele in the array of mating types potentiated, but perhaps differing from it in the probabilities of expressing the different mating types. An evaluation of this possibility required further study.

*The genetic analysis of the Alp-4 derivatives*

Family A produces five mating types, I, II, III, V and VI; under "standard" conditions the frequencies of caryonides manifesting these types are, respectively, 25%, 17%, 17%, 9% and 30%. The derived Alp-4 strains produce the same types but in very different frequencies, i.e., 60%, 12%, 9%, 0.3% and 20%. Such differences could be due to differences at the *mt* locus, or they could reflect differences at one or more other genetic loci. This question is susceptible to direct attack.

One could cross the Alp-4 derivatives with Family A and examine the $F_2$ segregants to determine whether the differences in frequency segregate as if controlled by a single pair of genes. This approach offers difficulties, since the arrays of types produced by the two series are identical, and an unambiguous classification of *mt* genotypes in the $F_2$ would require very large numbers, even assuming that they are distinctive. Fortunately, a simpler procedure is possible. Previous studies (NANNEY, CAUGHEY and TEFANKJIAN 1955) have shown that Families A and B are not distinguished by factors other than the *mt* locus which sensibly affect mating type frequencies; the homozygous $F_2$ segregants derived from an intercross of Families A and B are indistinguishable from the original Family A or Family B homozygotes. Hence, a demonstration of differences in supplementary genetic factors between the derived Alp-4 strains and Family B is equivalent to a demonstration of differences between the Alp-4 strains and Family A; and most importantly, the $F_2$ *mt* classes are much easier to identify.

Hence, a strain of the derived Alp-4 series ($F_4$) was crossed to an inbred ($F_7$) representative of Family B. Three of the first generation pairs from this cross were chosen to produce the $F_2$ by crosses of sister caryonides. Sixty-four of the viable true-conjugant pairs were then subjected to study for mating type potentialities. The first task was to verify the control of the mating type array by a single locus.

Certain simplifying assumptions were introduced to minimize the work involved in assigning potentialities. In previous studies the ability to produce type I and types IV and VII behaved as alternatives. Some $F_2$ pairs failed to produce type

I, some failed to produce types IV and VII, and some produced all three types. Since IV and VII potentialities had not been found to dissociate in previous crosses, their inseparability in these crosses was assumed. Thus, if type IV appeared, the potentiality for type VII was considered to be present, and *vice versa*. The adoption of this assumption prevents the use of these data in estimating the frequency of separation of the components of a possibly compound locus. It makes possible on the other hand, the assignments of Family B alleles to the $F_2$ without the exhaustive tests sometimes required before the relatively rare type VII appears.

The second assumption was that types I and IV would appear in reasonably high frequencies or not at all. This assumption was also based on the previous studies which indicated that these types were either common (10 percent or more) or absent. The importance of the assumption lies in its use in classifying certain $F_2$ pairs without progeny tests, using only the mating types expressed in the four caryonides of the $F_2$. Thus, if among the caryonides of a single pair both types I and IV, or types I and VII were observed, the pair was classified as a heterozygote. Fifteen of the 64 pairs were of this type.

If type I did not appear in the $F_2$, or if neither IV nor VII appeared in the $F_2$, further tests were required. Whenever possible these consisted of crosses of sister caryonides to yield an $F_3$. In some cases, however, all the $F_2$ caryonides were alike in mating type and could not be crossed; in other pairs only one of the four caryonides survived; even in some cases where crosses between sister caryonides were possible, all or most of the progeny died. In most such instances backcrosses could be carried out. If neither type I nor types IV or VII appeared in the $F_2$, backcrosses to both parents were required, but if type I was present only the backcross to the Alp-4 derivative was necessary; if either type IV or VII was present, only the cross to Family B was made.

Of the 64 pairs studied, 16 produced neither IV nor VII in progeny tests which usually involved from 20 to 60 caryonides; these all produced type I. Twelve failed to produce type I in samples of similar size, but did produce IV and, in most cases, VII. Thirty-four produced both I and IV or VII; these include the fifteen pairs classified without progeny tests. Two pairs were sterile both on inbreeding and in backcrosses; both of these pairs, however, included a type I caryonide. None of the 64 segregants was negative for I, IV and VII. The appearance of the "double negative" would be expected on a two-factor basis, and its absence —as in the crosses between Families A and B—supports the interpretation that the I and IV–VII determinants are allelic factors. Moreover, the 16:34:12 ratio in the $F_2$ is consistent with the 1:2:1 ratio expected on a single factor hypothesis. Hence, we may assume that one of the *mt* alleles had been extracted from Alp-4 and that it carried with it the potentiality for mating type I.

The question remains, however, whether the frequencies of the mating types, or even the potentialities for the other mating types, are determined in whole or in part by the *mt* locus. Conceivably the *mt* allele in Alp-4 is actually the same allele found in Family A, and the striking differences in mating type frequencies

are determined by genes at other loci. An analysis of the frequencies of the mating types produced in the progeny tests of the $F_2$ segregants permits a partial answer to these questions.

If Alp-4 and Family B differ in supplementary factors, they should segregate in the $F_2$, in part at least independently of the *mt* locus. The recovered Alp-4 homozygotes should differ from the original inbred parents, and the recovered Family B homozygotes should differ from their inbred parents. Such differences should be manifested in mating type frequencies in progeny tests, both in backcrosses and to a greater extent in $F_3$ progeny tests. The pooled data are presented in Table 4. They demonstrate significant differences between the parental homozygotes and the corresponding $F_2$ homozygotes. Specifically, when the Family B mating type allele is combined with factors from Alp-4, the frequency of type IV increases and that of type VI declines. When the Alp-4 allele is combined with factors from Family B, the frequency of type I declines and the frequency of type VI rises. Less striking differences may occur with other mating types. Since these modifications in mating type frequencies were detected with a limited number of $F_2$ pairs, and since these pairs are probably not a random sample of $F_2$ segregants, little can be said about the magnitude of the differences between Alp-4 and Family B. The two series do, however, differ in factors other than the *mt* genes which affect mating type frequencies.

Some additional information regarding the supplementary factors might be obtained by analyzing the variations among the progeny tests from different $F_2$ pairs. Although the sample sizes are too small for detailed quantitative analysis, borderline values in significance tests for homogeneity have been found. The available data are not adequate for an estimation of the number of additional genes segregating.

The evidence for supplementary factors does not apply apparently to mating type V. The vanishingly small fraction of this type in both the original Alp-4 line and in the recovered *mt* homozygotes strongly suggests that the factor re-

TABLE 4

*Comparison of mating type frequencies in crosses in which the parents have the same genes at the* mt *locus, but in which the parents may differ in other genes segregating in the cross between Family B and Alp-4 derivatives*

| Parents | Mating types of progeny | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII | |
| Inbred Family B | .000 | .149 | .093 | .473 | .045 | .139 | .100 | 1090 |
| $F_2$ (B) Backcross | .000 | .123 | .123 | .556 | .035 | .082 | .071 | 171 |
| $F_2$ (B) Intercross | .000 | .179 | .052 | .553 | .043 | .069 | .107 | 307 |
| | Chi-square: 28.5 | | D.F.: 10 | | P: $<.01$ | | | |
| Inbred Alp-4 | .596 | .119 | .087 | .000 | .003 | .198 | .000 | 356 |
| $F_2$ (Alp-4) Backcross | .454 | .113 | .088 | .000 | .005 | .321 | .000 | 218 |
| $F_2$ (Alp-4) Intercross | .498 | .145 | .045 | .000 | .002 | .312 | .000 | 401 |
| | Chi-square: 20.1 | | D.F.: 6 | | P: $<.01$ | | | |

sponsible is either closely linked to or equivalent to the Alp-4 *mt* allele. Hence, all the differences between Alp-4 and Family A may not be due to supplementary genetic factors; some could be due to differences at the *mt* locus itself. At least one must conclude that the Alp-4 chromosome is different from the Family A chromosome. This chromosome could also have significantly different effects on other mating types, but this has not been established.

## DISCUSSION

### *The designation of genotypes*

The observations just reported raise certain terminological problems. The two alleles at the *mt* locus previously recognized were given descriptive designations; the Family A allele was called $mt^{(IV,VII)-}$ and the Family B allele was called $mt^{(I)-}$, to indicate the types which were not produced when the alleles were homozygous. The allele derived from Alp-4 is identical with the Family A allele in the array of types which it permits, but it may differ in the probability of expression of those types. Fully descriptive allelic designations would require not only the mating type array, but also the frequencies of expression of the various mating types under standard conditions of residual genotype and environment. The information necessary for such designations is not yet available and the symbols would, in any case, be too cumbersome for manipulation. Since all the information about the effect of an allele cannot be put into its designation, a simpler but less informative designation will be employed in future work. The allele in Family A will be designated as $mt^A$; that in Family B will be called $mt^B$. The derived Alp-4 strains will be designated as Family C and the allele which they carry will be called $mt^C$. Similarly, the derived UM-226 strains will be called Family D and their allele, $mt^D$.

This study suggests that a number of genes influence the frequencies with which mating types appear. Thus far, however, no evidence indicates that loci other than the *mt* locus determine the array of types which can be expressed. The significance of the genetic background must be recognized, even though the mode of action of the additional genes is not yet understood. For the present, the genetic background may be described as a series of "polygenic complexes", apparently fixed in Families A, B and C, but still labile in Family D. Since Families A and B seem to have equivalent backgrounds and arose from a common source, their complex may be called the Massachusetts-1 complex. Family C, although derived from an intercross between a Michigan and a Massachusetts strain, appears to have fixed a distinctive set of supplementary factors, which will be referred to as the Michigan-1 complex. If a true-breeding series can be established in Family D, the background will be called the Vermont-1 complex.

The data now available relating genotypes to the frequencies of mating types produced at conjugation at 26°C in variety 1 are summarized in Table 5. All the data for Family A are not included; in particular, the Family A clones produced after a long series of inbreeding generations (NANNEY 1957) have been excluded since they show slightly different mating type frequencies than the original

TABLE 5

*Summary of data relating genotype and mating type frequencies within inbred strains of variety 1,* Tetrahymena pyriformis

| Genotypes of progeny | Mating type frequencies | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII | |
| $mt^A/mt^A$ (Mass.1/Mass.1) | .253 | .173 | .174 | .000 | .095 | .304 | .000 | 809 |
| $mt^A/mt^B$ (Mass.1/Mass.1) | .116 | .136 | .146 | .236 | .070 | .179 | .121 | 399 |
| $mt^B/mt^B$ (Mass.1/Mass.1) | .000 | .149 | .093 | .473 | .045 | .139 | .100 | 1090 |
| $mt^B/mt^C$ (Mass.1/Mich.1) | .215 | .116 | .071 | .260 | .007 | .296 | .050 | 284 |
| $mt^C/mt^C$ (Mich.1/Mich.1) | .596 | .119 | .087 | .000 | .003 | .198 | .000 | 356 |
| $mt^C/mt^A$ (Mich.1/Mass.1) | .245 | .208 | .107 | .000 | .044 | .396 | .000 | 159 |

Family A clones. These differences may account for the slight heterogeneity noted when all Family A progenies are compared (NANNEY 1956) and probably reflect some residual genetic heterogeneity within the clones from which the highly inbred strains were derived.

(Since this manuscript was submitted for publication, information from two independent studies has suggested that a clerical error, possibly at the $F_1$ level, was involved in the derivation of Families C and D. Specifically, the esterases (ALLEN unpublished) and the serotypes (LOEFER unpublished) of Family C resemble those of UM-226, not those of Alp-4. Analyses of differences among the inbred strains would not be affected by such considerations, but in any future attempt to derive similar strains, the possible misidentification of origin of Families C and D should be noted.)

### *The number of mating types within a variety*

Several apparently different patterns of mating type determination occur in the various species of Paramecium and Tetrahymena, but they all show peculiarities which are difficult to explain in conventional genetic terms, and they all agree in certain significant features. Before work was initiated on Tetrahymena, regularities were observed in the numbers of mating types found within varieties of Paramecium (See METZ 1954; SONNEBORN 1957). Only two mating type specificities are known to occur within a variety of *P. aurelia*, *P. multimicronucleatum* or *P. caudatum*, but multiple mating types are common in *P. bursaria*. One variety of *P. bursaria* contains only two types, but three varieties contain four types and one contains eight types. This regularity in numbers of mating types, corresponding to a geometric progression, $2^n$, suggested an explanation for the basis of mating type specificity which involved complementary pairs of substances, one pair in a two-type system, two pairs in a four-type system and three pairs in an eight-type system. This explanation received support from other observations and is an important suggestion concerning the chemical basis for mating behavior in these ciliates. If the hypothesis of paired complementary substances has general applicability, then all systems of multiple mating types in these ciliates should contain numbers of mating types fitting the $2^n$ progression.

Such regularities in numbers of mating types have not been demonstrated, however, in Tetrahymena. Of the nine varieties now known, two have two mating types, three have three mating types, one each has five, seven, eight and nine mating types (GRUCHY 1955; ELLIOTT and HAYES 1955; HURST 1958; ORIAS 1958). The failure to demonstrate a geometrical progression is not yet critical. Collections of some varieties are very few in number and almost certainly do not contain representatives of all the possible mating types. Moreover, only three varieties have been subjected to intensive breeding analysis; such breeding tests may increase the known number of mating types in some varieties. Nevertheless, none of the three most intensively studied varieties has produced a number of types in agreement with the hypothesis.

The study just reported yields a pessimistic prognosis for obtaining critical evidence on this problem in Tetrahymena. Certainly all the types possible within a variety cannot be obtained from crossing any two strains within the variety. Even when a type can be produced by a particular cross, its frequency may be very low (e.g., type V in Family C), and that type may not be found with limited sample sizes. The failure to find new types in additional wild strains of variety 1 does not rule out the existence of undiscovered types and does not constitute evidence sufficient to reject a geometrical progression. However, the data available from Tetrahymena provide no support for the universality of such progressions.

The observations on Tetrahymena cannot be disregarded because of the taxonomic distance which separates Tetrahymena from Paramecium. The pattern of mating type determination in variety 1 of *T. pyriformis* (NANNEY 1956) is essentially identical to that in variety 1 of *P. aurelia* (SONNEBORN 1947, 1957), and the pattern in variety 2 of *T. pyriformis* (HURST 1958) corresponds in major respects to that in variety 1 of *P. bursaria* (JENNINGS 1942). The problem of mating type determination in this group of ciliates must be approached as a single problem.

### *The mechanism of mating type determination*

This study extends the factual basis upon which an understanding of mating type determination must be built. The evidence for the primary significance of the $m^t$ locus in determining mating type arrays supplements that previously available in this variety (NANNEY, CAUGHEY and TEFANKJIAN 1955) and also that in variety 1 of *P. aurelia* (BUTZEL 1955). Unlike the possibly related systems controlling antigenic specificities in these ciliates (See BEALE 1954; SONNEBORN 1957) the factors potentiating alternative specificities are not distributed throughout the genome, but are localized on one particular chromosome and have not yet been separated, although in admittedly inadequate tests. This localization, which can scarcely be fortuitous, suggests that the persistent nuclear alterations involved in mating type determination may be alterations at, or near, these chromosomal sites. Certain other facts support the opinion that the nuclear alterations are, in fact, chromosomal alterations of some kind (NANNEY 1958). In any case, one need not postulate long routes of communication between genes controlling

mutually exclusive potentialities. As yet no judgment can be rendered concerning the complexity of the *mt* locus; each mating type might be represented by a genetically separable element or the mating type locus might be, in some functional sense, unitary. In the latter case, mating type determination might involve the establishment of some stable, but poorly understood "allelic configuration", the array of allelic states being dependent in some way on the allelic structure. For example, the DNA at the *mt* locus might determine the sequence of amino acids in a single protein molecule which is capable of several different folding configurations; materials or conditions in the vicinity of the locus might determine the patterns of folding and might be perpetuated through feed-back mechanisms.

The supplementary genetic factors may influence the establishment of different nuclear states by altering the intracellular environment. Effects similar to these genetic effects have been achieved by modifying the extracellular environment (SONNEBORN 1947; NANNEY unpublished) and the cytoplasmic environment in which the nuclei differentiate (SONNEBORN 1957; NANNEY 1954). The supplementary factors are of limited analytical use, however, until they can be defined in more specific terms and manipulated to show relationships between mating types.

## SUMMARY

New mating types have been sought in breeding experiments with wild strains of variety 1 of *T. pyriformis* from four natural sources in Michigan, Vermont and Massachusetts. No mating types beyond the seven derived from the original strains of variety 1 (WH-6 and WH-14) have been found.

Alleles at the *mt* locus have been extracted from two of the wild strains and established in homozygous condition in inbred series. The new inbred series are designated as Families C and D. They are similar to Family A, previously described, in producing mating types I, II, III, V and VI. Families A, C and D differ, however, in the frequencies of caryonides of the various types produced at conjugation. The differences between Families A and C are shown to depend in part on the chromosome bearing the *mt* locus and in part on other genetic factors.

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# NEW MARKERS AND MULTIPLE POINT LINKAGE DATA IN NEUROSPORA[1]

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LINKAGE maps of *Neurospora crassa* based on compiled tetrad data were published by BARRATT, NEWMEYER, PERKINS and GARNJOBST in 1954. Improved and extended maps based on new data have since been provided by MITCHELL and MITCHELL (1954) for group IV, and by STADLER (1956a) for group VI. The purpose of the present work has been to augment and improve the maps in order to provide suitably marked chromosomes for investigations related to crossing over.

It has become increasingly clear since 1954 that the intrinsic variability of crossover values for the same interval, in crosses of different parentage, may be so great that gene order cannot be established reliably by combining two-point data from heterogeneous sources (see especially STADLER 1956a). For this reason, the data presented here have been collected predominantly from multiple point crosses.

These data will be presented in a series of four papers. The present paper describes new markers that have been mapped in all seven groups, and presents segregation data for genes in groups I, II, VI and VII. Linkage data for markers in groups III, IV, and V are presented in the accompanying papers (PERKINS and ISHITANI 1959; MALING 1959b; STRICKLAND, PERKINS, and VEATCH 1959).

Fifty-three previously unmapped mutants have been localized in the seven established linkage groups. Among them are at least 25 new loci, which are mapped in relation to previously known genes. The remainder are recurrences. Several linkage groups have been extended by the addition of new terminal markers, and the sequences of a number of previously mapped loci have been clarified.

The data were obtained almost entirely from segregants collected by nonselective methods, as random segregants rather than as tetrads. Techniques are described that have made it easier to detect linkage and to obtain segregation data of the type desired.

## MATERIALS AND METHODS

### *Wild type and mutant strains*

ST. LAWRENCE wild type strains 74A and 73a, or derivatives from them, were used as standard parents for inbreeding and testing. Cytological studies of 74A

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TABLE 1

*Mutants previously unassigned to loci or linkage groups*

| Isolation number | Locus symbol, name, and linkage group | Origin: treatment and strain | Characteristics and remarks |
|---|---|---|---|
| B4 | *ro-1* : ropy-1* (IV) | U.V.; 74A | Cable-like aggregations of hyphae grow up tube from the agar. |
| STL4 | *lys-4* : lysine-4 (I) | spontaneous; 31873 | |
| STL6 | *un*(STL6) : unknown* (I) | U.V.; 39113 | Suboptimal response to methionine. Fluffyish morphology, late conidiation. |
| B6 | *bis* : biscuit* (V) | U.V.; 74A | Colonial. Flat at first, then sending up densely conidiating growth. |
| B9 | *ad-3* : adenine-3 (I) | U.V.; 74A | Accumulates purple pigment. |
| B12 | *bis* : biscuit* (V) | U.V.; 74A | See B6. |
| B15 | *ro-1* : ropy-1* (IV) | U.V.; 74A | See B4. |
| M16 | *os* : osmotic (I) | U.V.; B53, B123 | See B135. |
| B18 | *vel* : velvet* (III) | U.V.; 74A | A soft, conidiating colonial. |
| B20 | *ro-2* : ropy-2* (III) | U.V.; 74A | Phenotypically indistinguishable from *ro-1* (B4). |
| B30 | *bis* : biscuit* (V) | U.V.; 74A | See B6. |
| B40 | *bn* : button* (VII) | U.V.; 74A | Nonconidiating restricted colonial growth. |
| B53 | *rg* : ragged* (I) | U.V.; 74A | Poorly conidiating colonial growth. Small, densely conidiating, pigmented, *cr rg* colonies are useful for plating and replication (MALING 1959a). |
| B54 | *com* : compact* (III) | U.V.; 74A | Small colonies. |
| B56 | *bal* : balloon* (II) | U.V.; 74A | Restricted growth on agar as a hemispheric colony. A good protoperithecial parent. |
| B57 | *mat* : mat* (IV) | U.V.; 74A | Colonial. Prefers sucrose to glycerol. |
| B66 | *wl* : woolly* (V) | U.V.; 74A | Conidiation variable. Probably associated with an aberration. |
| B74 | *cr* : crisp (I) | U.V.; 74A | See B123. |
| B102 | *al* : albino (I) | U.V.; 74A | |
| B105p | *ad-3* : adenine-3 (I) | U.V.; 74A | Accumulates purple pigment. |
| B106 | *sk* : skin* (VII) | U.V.; 74A | Leathery nonconidiating rapid growth hugs agar surface. |
| B110 | *fr* : frost* (I) | U.V.; 74A | Delicate branching on surface, and delicate aerial growth. Nonconidiating. |
| B118 | *pl* : plug* (V) | U.V.; 74A | Characteristic dense hyphae fill diameter of 10 mm tube. |
| B122 | *cr* : crisp (I) | U.V.; 74A | See B123. Suspected aberration. |
| B123 | *cr* : crisp (I) | U.V.; 74A | Early conidiation, uniform over agar surface. |
| B128 | *st* : sticky* (I) | U.V.; 74A | Difference from wild type subtle but can be scored. |
| B132 | *sp* : spray* (V) | U.V.; 74A | Characteristic surface growth followed by growth upward and outward. |

| Isolation number | Locus symbol, name, and linkage group | Origin: treatment and strain | Characteristics and remarks |
|---|---|---|---|
| B135 | *os* : osmotic (I) | U.V.; 74A | Sensitive to high osmotic pressure. Can be scored by appearance. Conidia rare except in combination with crisp. |
| B137 | *del* : delicate* (VI) | U.V.; 74A | Growth less profuse than wild type. |
| B148 | *col-4* : colonial-4 (IV) | U.V.; 74A | Dense balls of conidia high in slant. |
| B149 | *slo* : slow* (I) | U.V.; 74A | Normal morphology achieved days later than wild type. Not tested for allelism with *cyt*(C115). |
| M155-5 | *fl* : fluffy (II) | H263R4A×H263R3a | |
| B180 | *cr* : crisp (I) | U.V.; 74A | Least flat and restricted of the crisp alleles. |
| B187 | *rg* : ragged* (I) | U.V.; 74A | See B53. |
| JH216 | *al* : albino (I) | N-mustard | |
| B230s | *so* : soft* (I) | X-ray; 74A | Resembles peach. Dense, delicately pigmented growth fills lower part of slant. |
| B233t | *ti* : tiny* (I) | X-ray; 74A | Small dense colony, with gnarled, short hyphae. |
| B234 | *sk* : skin* (VII) | X-ray; 74A | Probable allele of B106, which it resembles. (Intercrosses are not fertile.) |
| P346 | *fl* : fluffy (II) | 51504A×Pa | |
| Y602 | *al* : albino (I) | N-mustard; 1A×25a | |
| P605 | *fl* : fluffy (II) | Spontaneous; 74A background | |
| Y2170 | *al* : albino (I) | N-mustard; 1A×25a | |
| Y2171 | *al* : albino (I) | N-mustard; 1A×25a | |
| 4894 | *me-7* : methionine-7* (VII) | X-ray; LA×La | Uses homocysteine, not cysteine.. One unit from centromere (Buss 1944). |
| JH9698 | *al* : albino (I) | N-mustard | |
| 27947 | *arg-5* : arginine-5* (II) | X-ray; 1A×19a | Uses ornithine, citrulline, or arginine. |
| 30820 | *arg-11* : arginine-11* (VII) | X-ray; 1A×19a | Requires citrulline or arginine plus a purine plus a pyrimidine. |
| Y31881 | *nic-3* : nicotinic-3* (VII) | N-mustard; Y8743 | Uses 3-hydroxyanthranilic acid. |
| 37501 | *leu*(37501) : leucine* (IV) | U.V.; 1A×25a | More stable than possible allele 8839. (D. Regnery, unpub.). |
| 45201 | *val* : valine* (V) | U.V.; Abb4×25a | No isoleucine requirement. First two digits of isolation number possibly in error. |
| $al^C$ | *al* : albino (I) | X-ray; B53, B123 | |
| $al^M$ | *al* : albino (I) | U.V.; B53, B123 | |
| $fl^P$ | *fl* : fluffy (II) | Spontaneous; 74A background | |

* Asterisks designate previously unmapped loci. Mutants are arranged in numerical order of their isolation numbers, without regard to letter prefixes.

10 μg per ml (except inositol, 30 μg per ml); amino acids, purines and pyrimidines: 0.1 mg or 0.2 mg per ml (except arginine, 0.5 mg L-arginine HCl per ml).

For optimal growth arginine mutants generally require the addition of arginine even to complete medium. 0.1 mg/ml L-arginine HCl was used for crossing (NEWMEYER 1957).

For optimal growth arginine-11 (30820) (SRB 1950) requires arginine (or citrulline) plus *both* a purine *and* a pyrimidine (NEWMEYER, personal communication). 0.3 mg/ml L-arginine, 0.2 mg/ml adenylic acid, and 0.1 mg/ml uridine have proved adequate for good germination and growth.

button *(bn)* germinates and survives better on minimal medium than on complete. $bn^-$ segregants can be scored for nutritional traits by inoculating on plates (25 or more tests per plate), but false negative readings may result unless inocula are checked microscopically for viability.

Ascospores from crosses segregating histidine or homoserine requirements were isolated to specifically supplemented minimal medium in order to avoid inhibition by constituents of complete medium (LEIN, MITCHELL and HOULAHAN 1948; HAAS, MITCHELL, AMES and MITCHELL 1952; TEAS, HOROWITZ and FLING 1948).

nitrate *(nit)* was scored visibly using a pH indicator (FINCHAM, personal communication). When one percent $KNO_3$ and 20 μg/ml brom-cresol purple are included in the medium, $nit^-$ turns the medium yellow after growth is complete, whereas $nit^+$ turns it purple.

Growth of osmotic *(os)* segregants from germinating ascospores is inhibited markedly by glycerol but not by one percent sucrose (ST. LAWRENCE, personal communication). osmotic ordinarily can be scored by appearance, but can be checked using medium to which four percent NaCl has been added. The mutant cannot grow under these conditions.

skin *(sk)* ascospores mature somewhat more slowly than $sk^+$, but good allele ratios were obtained at three weeks or longer after fertilization.

Germination of ascospores was good only below 30°C in the case of tiny, and probably of *amyc* as well.

Either indole (10 μg per ml) or DL-tryptophan plus DL-phenylalanine (0.2 mg of each per ml) was used for tryptophan mutants.

Scoring for mating type was accomplished in Petri dishes using wild types *A* and *a* as protoperithecial tester parents. The testers were inoculated into separate plates containing 12–15 ml of synthetic cross medium. After six days the isolates to be tested were inoculated at marked spots on plates containing each mating type. Twenty-four or more isolates can be tested on a single pair of plates. Negative tests were not trusted without confirmation against the other mating type.

## PRESENTATION OF DATA

Newly mapped mutants in all seven groups have been listed in Table 1. Linkage data with markers in groups I, II, VI and VII are presented in Tables 2 and 3, and summarized in Figure 1. The format of the tables was adapted from EMERSON,

TABLE 2

*Two and three point crosses with markers in groups I, II, VI, and VII*

| Zygote genotype and recombination percent | Parental combinations | | Recombinations: Singles region 1 | | Singles region 2 | | Doubles regions 1 and 2 | | Total and percent germination | Marker isolation numbers |
|---|---|---|---|---|---|---|---|---|---|---|
| + *b* *c*<br>*a* + + | +*bc* | *a*++ | +++ | *abc* | +*b*+ | *a*+*c* | ++*c* | *ab*+ | ... | (a)<br>(b)<br>(c) |
| + + *arg-1*<br>*fr* *leu-3* +<br>25.3 22.9 | 48 | 49 | 14 | 17 | 12 | 15 | 4<br>C=1.1<br>(>0.57) | 7 | 166<br>83% | B110<br>47313<br>B369 |
| + *leu-3* *cr*<br>*fr* + +<br>31.0 31.0 | 12 | .. | 8 | .. | 8 | .. | 1<br>C=0.4 | .. | 29<br>(*fr*$^+$ only)<br>60% | B110<br>47313<br>*cr*$^L$ |
| + *rg* +<br>*fr* + *aur*<br>26.3 36.8 | 9 | 7 | 4 | 4 | 5 | 7 | 0<br>C=0.5 | 2 | 38<br>38% | B110<br>B187<br>34508 |
| + *ad-3* +<br>*fr* + *aur*<br>27.0 24.3 | 9 | 12 | 4 | 3 | 2 | 4 | 0<br>C=1.2<br>(>0.26) | 3 | 37<br>92% | B110<br>B105p<br>34508 |
| + *cr* *aur*<br>*fr* + +<br>40.0 48.0 | 10 | .. | 3 | .. | 5 | .. | 7<br>C=1.5<br>(>0.58) | .. | 25<br>(*fr*$^+$ only)<br>92% | B110<br>*cr*$^L$<br>34508 |
| + + +<br>*fr* *cr* *aur*<br>50.0 58.3 | 3 | 2 | 3 | 2 | 5 | 2 | 7<br>C=1.0 | 0 | 24<br>100% | B110<br>*cr*$^L$<br>34508 |
| + + +<br>*fr* *cr* *aur*<br>43.0 31.4 | 24 | 10 | 14 | 11 | 8 | 7 | 5<br>C=1.0 | 7 | 86<br>86% | B110<br>*cr*$^L$<br>34508 |
| + *un* +<br>*fr* + *aur*<br>34.6 6.4 | 25 | 23 | 15 | 10 | 2 | 1 | 2<br>C=1.2<br>(>0.14) | 0 | 78<br>78% | B110<br>STL6<br>34508 |
| + *al-2*(*lys-3*)*os*<br>*fr* +(+)+<br>39.2 24.7 | 25 | 13 | 24 | 11 | 8 | 13 | 3<br>C=0.3 | 0 | 97<br>97% | B110<br>15300<br>(4545)<br>E11200 |
| + *arg-1* +<br>*leu-3* + *cr*<br>15.8 3.9 | 36 | 25 | 6 | 6 | 3 | 0 | 0 | 0 | 76$^N$<br>62% | 47313<br>46004<br>*cr*$^L$ |
| + *a*<br>*amyc* *A*<br>5.5 | .. | 17 | .. | 1 | .. | .. | .. | .. | 18<br>(*amyc*$^-$ only) | K422<br>sex |
| *A* + *arg-1*<br>*a* *ad-5* +<br>6.2 0 | 19 | 26 | 2 | 1 | 0 | 0 | 0 | 0 | 48$^N$<br>96% | sex<br>71104<br>46004 |
| *A* *arg-1* *ti*<br>*a* + +<br>14.3 0 | 18 | .. | .. | 3 | .. | 0 | 0 | .. | 21<br>(*ti*$^-$ only)<br>76% | sex<br>46004<br>B233t |

TABLE 2—Continued

*Two and three point crosses with markers in groups I, II, VI, and VII*

| Zygote genotype and recombination percent | Parental combinations | | Recombination: Singles region 1 | | Singles region 2 | | Doubles regions 1 and 2 | | Total and percent germination | Marker isolation numbers |
|---|---|---|---|---|---|---|---|---|---|---|
| (+) *A arg-1* + / (*leu-3*) *a* + *cr* (1.8, 5.5) | 49 | 52 | 0 | 2 | 2 | 4 | 0 | 0 | $109^{N}$ 56% | (47313) sex 46004 $cr^{L}$ |
| *A ti* / *a* + (7.1) | 13 | .. | .. | 1 | .. | .. | .. | .. | 14 (*ti*⁻ only) 83% | sex B233t |
| *A aur* + / *a* + *so* (26.8, 31.4) | 20 | 14 | 7 | 5 | 7 | 8 | 5 1 C=1.1 (>0.39) | | 67 96% | sex 34508 B230s |
| (*A*) + *arg-1* *cr* / (*a*) *un* + + (2.3, 11.6) | 57 | 54[a] | 2 | 1[a] | 11 | 4[a] | 0 | 0 | $129^{MA}$ 64% | (sex) 55701t 46004 $cr^{L}$ |
| + *arg-1* *cr* / *ad-5* + + (0, 2.9) | 14 | 20 | 0 | 0 | 0 | 1 | 0 | 0 | $35^{N}$ 70% | 71104 46004 $cr^{L}$ |
| + *rg* *cr* / *ad-5* + + (10.2, 13.8) | 50 | 80 | 11 | 3 | 9 | 11 | 2 1 C=1.3 (>0.26) | | 167 82% | 71104 B53 B123 |
| (a) + *ti* (+) / (A) *arg-1* + (*cr*) (1.6) | 61 | .. | .. | 1[b] | .. | .. | .. | .. | 62 (*ti*⁻ only) 88% | (sex) 46004 B233t ($cr^{L}$) |
| (a) + *lys-4* + / (A) *rg* + *cr* (6.6, 9.0) | 104 | 36 | 8 | 3[A] | 7[a] | 8 | 0 | 0 | 166 58% | (sex) B53 15069 B123 |
| (A) + + + / (a) *rg* *lys-4* *cr* (1.3, 1.3) | 43 | 35 | 1[A] | 0 | 0 | 1[a] | 0 | 0 | 80 80% | (sex) B53 15069 B123 |
| + + (*arg-6*) *al-2* / *rg* *cr* (+) + (14.3, 33.3) | 5 | 6 | 1 | 2 | 4 | 3[a] | 0 | 0 | 21 52% | B53 B123 (29997) 15300 |
| + + *al-2* (*hs*) / *rg* *cr* + (+) (16.7, 38.1) | 10 | 12 | 1 | 3 | 9 | 4[g] | 0 3 C=1.1 (>0.23) | | 42 70% | B53 B123 15300 (51504) |
| + + *al* / *rg* *cr* + (9.7, 45.1) | 41 | 43 | 4 | 8 | 33 | 41 | 2 3 C=0.7 | | 175 79% | B53 B123 4637T |
| + + *aur* (*hs*) / *rg* *cr* + (+) (5.3, 44.7) | 12 | 8 | 0 | 1 | 10 | 6[g] | 0 1 C=1.1 (>0.03) | | 38 63% | B53 B123 34508 (51504) |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| + *cr* + / *rg* + *aur* (2.9, 29.4) | 21 | 25 | 1 | 1 | 10 | 10 | 0 | 0 | 68 / 68% | B187 B123 34508 |
| + + *(arg-6) aur* / *rg* *cr* (+) + (0, 47.2) | 15 | 4 | 0 | 0 | 7 | 10[f] | 0 | 0 | 36 / 90% | B53 B123 (29997) 34508 |
| + *st* + / *ad-3B* + *thi-1* (4.6, 14.0) | 43 | 27 | 1[A] | 3[i] | 7[A] | 5[a] | 0 | 0 | 86 / 86% | 35203 B128 56501 |
| + *thi-1* + / *ad-3B* + *al-2* (20.3, 17.4) | 44 | 45 | 15 | 10 | 11 | 10 | 2 1 C=0.6 | | 138 / 69% | 35203 56501 15300 |
| + + + / *cr* *thi-1* *me-6* (11.1, 12.4) | 34 | 28 | 7 | 2 | 3 | 7 | 0 | 0 | 81 / 81% | *cr*[L] 56501 35809 |
| + + *me-6* / *slo* *thi-1* + (5.4, 14.3) | 49 | 42 | 2 | 3 | 7 | 8 | 1 0 C=1.2 (>0.03) | | 112 / 81% | B149 56501 35809 |
| + *thi-1* *nit* / *slo* + + (2.2, 28.6) | 33 | 30 | 0 | 2 | 13 | 13 | 0 | 0 | 91 / 91% | B149 56501 34547 |
| *(a)* + *me-6* / *(A)* *slo* + (15.5) | 19 | 19 | 2[a] | 5[A] | .. | .. | .. | .. | 45 / 90% | (sex) B149 35809 |
| + + *al-2* / *slo* *me-6* + (17.8, 28.8) | 25 | 15 | 8 | 4 | 11 | 9 | 0 1 C=0.3 | | 73 / 73% | B149 35809 15300 |
| + *nit* *aur* / *slo* + + (39.4, 21.3) | 18 | 11 | 9 | 10 | 3 | 5 | 1 4 C=1.0 | | 61 / 79% | B149 34547 34508 |
| + + *nit* / *thi-1* *me-6* + (9.6, 6.4) | 83 | 75 | 8 | 10 | 8 | 4 | 0 | 0 | 188 / 95% | 56501 35809 34547 |
| + + *al-2* / *thi-1* *me-6* + (13.5, 26.9) | 19 | 13 | 3 | 3 | 5 | 8 | 1 0 C=0.5 | | 52 / 87% | 56501 35809 15300 |
| + *mac* *aur* / *thi-1* + + (7.1, 17.9) | 34 | 30 | 2 | 3 | 6 | 8 | 1 0 C=0.9 | | 84 / 84% | 56501 65108 34508 |
| (+) + *me-1* / *(thi-1)* *me-6* + (48.0) | .. | .. | 13 | .. | .. | .. | .. | .. | 54 / 90% | (56501) 35809 38706 |
| + *un* *aur* / *nit* + + (5.0, 7.5) | .. | 35 | 2 | .. | .. | 3 | 0 | .. | 40 (*un*[+] only) >80% | 34547 STL6 34508 |
| *(a)* + *al-2* / *(A)* *arg-6* + (0.4) | .. | 243 | .. | 1[A] | .. | .. | .. | .. | 244[s] (*arg*⁻ only) | (sex) 29997 15300 |

TABLE 2—Continued

*Two and three point crosses with markers in groups I, II, VI, and VII*

| Zygote genotype and recombination percent | Parental combinations | Recombination: Singles region 1 | Recombination: Singles region 2 | Recombination: Doubles regions 1 and 2 | Total and percent germination | Marker isolation numbers |
|---|---|---|---|---|---|---|
| (a) + *aur* / (A) *arg-6* + 0.4 | .. 260 | .. 1[A] | .. .. | .. .. | 261[s] (*arg*⁻ only), | (sex) 29997 34508 |
| + + *hs* / *arg-6* *aur* + | .. .. | 5 .. | 3 .. | .. .. | 8[s] (*arg*⁺*hs*⁺ only) | 29997 34508 51504 |
| (+ + *nit*) + *aur* (+ *nic-1*) / (*cr thi-1* +) *al-2* + (*lys-3* +) 4.2 | .. .. | 1[d] .. | .. .. | .. .. | 48 80% | ($cr^L$) (56501) (34547) 15300 34508 (4545) (3416) |
| (a) + *hs* / (A) *al-2* + 2.5 | 77 .. | .. 2[h] | .. .. | .. .. | 79[s] (*hs*⁻ only) | (sex) 15300 51504 |
| + *hs* / $al^M$ + 0 | .. 76 | .. 0 | .. .. | .. .. | 76[B] (*al*⁻ only) 75% | $al^M$ 51504 |
| (a) + *hs* / (A) *aur* + 0.4 | 277 .. | .. 1[a] | .. .. | .. .. | 278[s] (*hs*⁻ only) | (sex) 34508 51504 |
| + *os* + / *aur* + *so* 26.9 7.7 | 35 41 | 27 17 | 4 5 | 1 0 C=0.4 | 130 87% | 34508 B135 B230s |
| + *os* *so* / *nic-1* + + 28.2 6.0 | 30 48 | 22 10 | 5 1 | 1 0 C=0.5 | 117 78% | 3416 B135 B230s |
| + + + (+) / *bal* *arg-5* *pe* (*fl*) 11.1 33.3 | 5 .. | 1 .. | 3 .. | 0 .. | 9 (*bal*⁺ only) 50% | B56 27947 Y8743m ($fl^L$) |
| + *arg-5* (*pe fl*) / *bal* + (+ +) 1.2 | 35 47 | 0 1[c] | .. .. | .. .. | 83 83% | B56 27947 (Y8743m) ($fl^L$) |
| *bal*-centromere / 0 | 29–1st division segregation asci | 0–2nd division segregation asci | | | 29 tetrads 100% | B56 centromere |
| (*bal*) + + + / (+) *arg-5* *pe* *fl* 28.6 31.4 | .. 15 | .. 9 | .. 10 | .. 1 C=0.3 | 35 (*bal*⁺ only) 83% | (B56) 27947 Y8743m $fl^L$ |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| + *pe* *fl*<br>*arg-5* + +<br>5.8 28.8 | 18 | 16 | 1 | 2 | 7 | 8 | 0 | 0 | 52<br>76% | 27947<br>Y8743m<br>$fl^L$ |
| + + +<br>*pe* *arom* *fl*<br>2.9 23.0 | 45 | 58 | 4 | 0 | 14 | 18 | 0 | 0 | 139<br>91% | Y8743m<br>Y7655<br>$fl^L$ |
| + *tryp-3*<br>*ac* +<br>11.9 | 22 | 15 | 3 | 2 | .. | .. | .. | .. | 42<br>70% | Y2492<br>C83 |
| + *asco* *(tryp-2)*<br>*del* + (+)<br>1.5 | .. | 64 | 1 | .. | .. | .. | .. | .. | 65[H]<br>(*asco*$^+$<br>only)<br>65% | B137<br>37402<br>(45302) |
| + *bn* +<br>*thi-3* + *arg-10*<br>0 23.9 | 36 | 34 | 0 | 0 | 13 | 9 | 0 | 0 | 92<br>88% | 18558<br>B40<br>B368 |
| + *bn* *arg-10*<br>*thi-3* + +<br>3.4 21.4 | 32 | 35 | 3 | 0 | 11 | 8 | 0 | 0 | 89<br>89% | 18558<br>B40<br>B317 |
| + + +<br>*bn* *arg-10* *nt*<br>26.8 7.3 | 28 | .. | 10 | .. | 2 | .. | 1<br>C=1.2<br>(>0.03) | .. | 41<br>(*bn*$^+$<br>only)<br>88% | B40<br>B370<br>65001 |
| + *arg-11* +<br>*bn* + *nt*<br>10.8 10.8 | 82 | 19 | 11 | 3 | 13 | 1 | 0 | 0 | 129[k]<br>66% | B40<br>30820<br>65001 |
| + *arg-10* +<br>*arg-11* + *nt*<br>1.6 1.6 | 97 | 84 | 1 | 2 | 1 | 2 | 0 | 0 | 187<br>94% | 30820<br>B317<br>65001 |
| + *arg-10* +<br>*arg-11* + *nt*<br>1.1 5.3 | 89 | .. | .. | 1 | .. | 5 | 0 | .. | 95<br>(*nt*$^+$<br>only)<br>95% | 30820<br>B317<br>65001 |
| + + *sk*<br>*arg-10* *nt* +<br>6.2 17.2 | 17 | 32 | 3 | 1 | 5 | 6 | 0 | 0 | 64<br>45% | B370<br>65001<br>B106 |
| + *nt* *sk*<br>*arg-10* + +<br>5.9 17.6 | 13 | .. | .. | 1 | .. | 3 | 0 | .. | 17<br>(*sk*$^-$<br>only)<br>82% | B370<br>C86<br>B106 |
| + + *sk*<br>*arg-10* *nt* +<br>12.3 6.8 | 24 | 35 | 4 | 5 | 5 | 0 | 0 | 0 | 73<br>73% | B317<br>65001<br>B234 |

Numbers of progeny are given in the body of the table. Progeny genotypes are not designated explicitly, but the genotype of each class can be determined from the order of presentation. The left-hand number of each pair of complementary classes represents the genotype that contains the plus allele of the left-most marker scored (or mating type A). Alleles shown in parentheses in the genotype column were not scored or recorded, nor were classes of segregants where dashes replace numbers. Regions are numbered from left to right, and isolation numbers in the last column are listed in the same order. Crosses are tabulated in a sequence corresponding to the position of their left-most markers in the linkage group. Where gene order is uncertain, one arbitrarily chosen order has been used consistently. C=coincidence. When C exceeds one, the minimum coincidence is also given from which the observed value does not deviate significantly (2.5 percent one-sided level).

[a] Mating type *a*.
[A] Mating type *A*.
[B] Data of Mr. Edward G. Barry.
[b] *A*--+ by progeny test.
[c] ---- by progeny test.
[d] Phenotypically ++—++—+.
[e] Two *arg*$^-$, one *arg*$^+$.
[f] Nine *arg*$^-$, one *arg*$^+$.
[g] *All hs*$^-$.
[H] Data of Mr. Jerry L. Howard.
[h] One *A*, one *a*.
[i] One *A*, two *a*.
[k] Plus 23 *bn*$^-$ not scored.
[MA] Data of Mrs. M. K. Allen.
[N] Data of D. Newmeyer.
[s] Germinated ascospores isolated selectively from minimal plates.

## TABLE 3

*Four- five- and six-point crosses. Conventions as in Table 2. All markers are in Linkage Group I. Coincidence values are based on pairs of crossovers both in double and in triple recombination classes*

| Zygote genotype<br>recombination percent<br>and marker isolation numbers | Parental combinations | Recombinations | | | | | | | Total and percent germination |
|---|---|---|---|---|---|---|---|---|---|
| | | Singles | | | | | Doubles | Triples | |
| | | Region 1 | Region 2 | Region 3 | Region 4 | Region 5 | (Regions in parentheses) | | |
| + *leu-3* *ad-3B* *al-2* *lys-3*<br>*fr* + + + +<br>39.5 4.6 34.9 20.9<br>B110, 47313, 35203, 15300, 4545 | 13 .. | 8 .. | 1 .. | 7 .. | 2 .. | .. .. | 5 ..<br>(1&3) | 4 ..<br>(1&4) | 2 ..<br>(3&4)<br>C=1.0 | 1 ..<br>(2,3,4) | 43<br>(*fr*$^+$ only)<br>85% |
| + + *cr* *thi-1*<br>*fr* *leu-3* + +<br>26.0 11.7 7.8<br>B110, 47313, *cr*$^L$, 56501 | 47 .. | 15 .. | 7 .. | 3 .. | .. .. | .. .. | 2 ..<br>(1&2)<br>C=0.9 | 3 ..<br>(1&3) | .. .. | 0 .. | 77<br>(*fr*$^+$ only)<br>80% |
| + *A* + +<br>*fr* *a* *cr* *aur*<br>16.7 4.2 29.2<br>B110, —, B180, 34508 | 14 .. | 2 .. | 1 .. | 5 .. | .. .. | .. .. | 2 ..<br>(1&3) | .. .. | .. .. | 0 .. | 24<br>(*fr*$^+$ only)<br>90% |
| + *A* *slo* +<br>*fr* *a* + *aur*<br>36.4 13.6 20.4<br>B110, —, B149, 34508 | 6 9 | 4 10 | 4 1 | 4 4 | .. .. | .. .. | 1 0<br>(1&2)<br>C=0.5 | 1 0<br>(1&3) | .. .. | 0 0 | 44<br>88% |
| *A* + *rg* *cr* *thi-1* *al-2*<br>*a* *arg-1* + + + +<br>10.6 2.1 2.1 17.0 19.2<br>—, 46004, B53, B123, 56501, 15300 | 15 10 | 1 2 | 0 1 | 1 0 | 3 4 | 4 4 | 1 0<br>(1&4) | 1 0<br>(1&5) | .. .. | 0 0 | 47$^N$<br>95% |
| + *rg* *cr* *thi-1* *al-2*<br>*arg-1* + + + +<br>2.0 2.0 7.8 23.5<br>46004, B53, B123, 56501, 15300 | 23 11 | 0 0 | 0 1 | 1 3 | 7 4 | .. .. | 1 0<br>(1&4) | .. .. | .. .. | 0 0 | 51$^N$<br>64% |
| + *ad-3B* + *thi-1* *al-2*<br>*rg* + *cr* + +<br>0 8.9 7.1 17.9<br>B53, 35203, B123, 56501, 15300 | 20 18 | 0 0 | 3 1 | 2 2 | 5 4 | .. .. | 0 1<br>(2&4) | .. .. | .. .. | 0 0 | 56<br>70% |
| + + + +<br>*ad-3A* *nit* *aur* *os*<br>16.9 12.1 24.2<br>35203, 34547, 34508, B135 | 37 36 | 6 6 | 4 4 | 9 10 | .. .. | .. .. | 1 0<br>(1&2)<br>C=1.6<br>(>0.43) | 2 1<br>(2&3)<br>C=1.7<br>(>0.60) | 2 3<br>(1&3) | 2 1 | 124<br>83% |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *(A)* + *cr* *thi-1* *al-2*<br>*(a) lys-4* + + +<br>2.4 4.7 29.4<br>(–), STL4, B123, 56501, 15300 | 20 | 34 | 2[A] | 0 | 4 | 0 | 8 | 17 | .. .. | .. .. | .. .. | .. .. | .. .. | .. .. | .. .. | .. .. | 0 | 0 | 85<br>42% |
| *(a)* + *un* + +<br>*(A)* *cr* + *thi-1* *al-2*<br>1.0 6.9 23.8<br>(–), B123, 44409t, 56501, 15300 | 32 | 38 | 0 | 1[A] | 4 | 2 | 12 | 11 | .. .. | .. .. | .. .. | .. .. | 1 0<br>(2&3)<br>C=0.6 | .. .. | .. .. | .. .. | 0 | 0 | 101<br>67% |
| + + + + + +<br>*cr* *thi-1* *nit* *aur* *nic-1* *os*<br>10.1 23.4 14.8 18.0 6.2<br>$cr^L$, 56501, 34547, 34508, 3416, B135 | 28 | 22 | 4 | 4 | 9 | 15 | 8 | 6 | 4 | 8 | 2 | 4 | 0 1<br>(1&3)<br>2 1<br>(2&4) | 3 0<br>(1&4)<br>2 0<br>(3&4)<br>C=0.6 | 1 1<br>(2&3)<br>C=0.5<br>0 2<br>(4&5)<br>C=1.4<br>(>0.17) | 0 1<br>(1,2,4)<br>C=0.3<br>for<br>1&2 | 0 | 1 | 128<br>85% |
| + + + + + +<br>*cr* *thi-1* *nit* *aur* *nic-1* *os*<br>10.4 28.8 6.4 4.0 25.6<br>$cr^L$, 56501, 34547, 34508, 3416, E11200 | 32 | 12 | 0 | 9 | 18 | 10 | 0 | 4 | 1 | 3 | 20 | 3 | 3 1<br>(1&5)<br>3 1<br>(2&5) | 1 2<br>(2&3)<br>C=1.3<br>(>0.27)<br>1 0<br>(3&5) | 1 0<br>(2&4) | | 0 | 0 | 125<br>83% |
| + + *nit* *aur*<br>*cr* *me-6* + +<br>13.4 3.0 29.8<br>$cr^L$, 35809, 34547, 34508 | 19 | 22 | 3 | 1 | 0 | 2 | 9 | 6 | .. .. | .. .. | .. .. | .. .. | 3 2<br>(1&3) | .. .. | .. .. | .. .. | 0 | 0 | 67<br>96% |
| + + + +<br>*cr* *nit* *aur* *os*<br>42.9 19.0 27.0<br>B122, 34547, 34508, B135 | 13 | 9 | 9 | 7 | 3 | 1 | 4 | 4 | .. .. | .. .. | .. .. | .. .. | 3 1<br>(1&2)<br>C=1.2<br>(>0.43) | 1 1<br>(2&3)<br>C=1.2<br>(>0.33) | 2 3<br>(1&3) | | 0 | 2 | 63<br>93% |
| + *nic-1* *os* *so*<br>*al* + + +<br>5.1 11.5 3.8<br>4637T, 3416, B135, B230s | 30 | 34 | 2 | 1 | 5 | 2 | 0 | 2 | .. .. | .. .. | .. .. | .. .. | 1 0<br>(1&2)<br>C=2.2<br>(>0.05) | 1 0<br>(2&3)<br>C=2.9<br>(>0.08) | .. .. | .. .. | 0 | 0 | 78<br>79% |
| + + + *so*<br>*aur* *nic-1* *os* +<br>11.3 9.7 1.6<br>34508, 3416, B135, B230s | 55 | 44 | 7 | 4 | 6 | 3 | 2 | 0 | .. .. | .. .. | .. .. | .. .. | 1 2<br>(1&2)<br>C=2.2<br>(>0.44, | .. .. | .. .. | .. .. | 0 | 0 | 124<br>88% |

[A] Mating type *A*.

[N] Data of D. NEWMEYER.

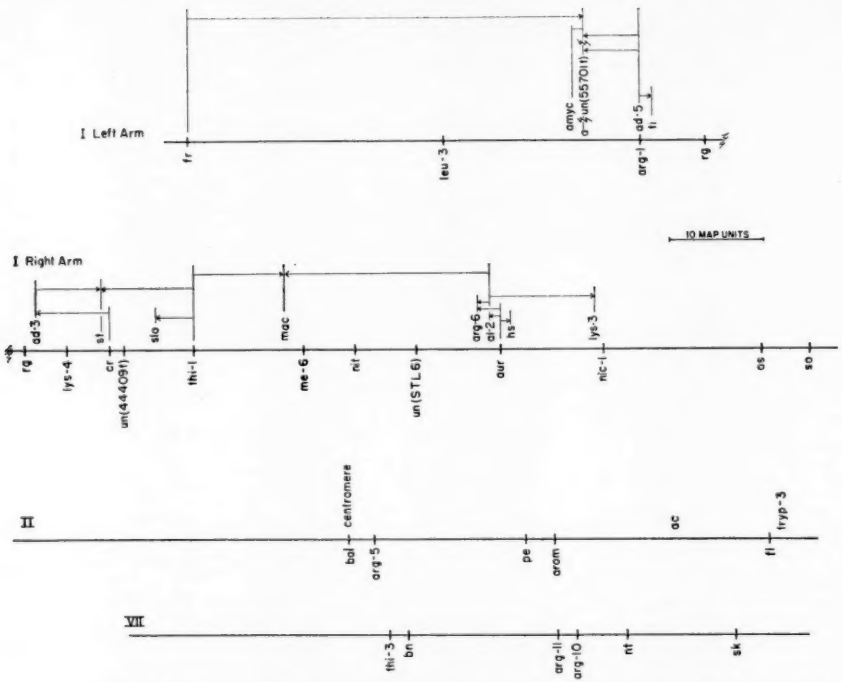


FIGURE 1.—Partial maps of groups I, II and VII summarizing the data from Tables 2 and 3. The sequence of loci written below the heavy line is based directly on 3-point data for all the genes involved. Above the heavy line, only the relationships indicated by arrows are based on 3-point data. Two opposing arrows indicate that the marker was situated medially in the 3-point cross; each unopposed arrow originates from a medial marker and leads to a marker that was not medial. Interval lengths are imprecise owing to the variability of crossing over. The map is based solely on data from the present paper, except that the position shown for genes above the line may reflect the results of other workers in cases where our own data would require order to be decided arbitrarily or to be based only on quantitative 2-point data from different sources. See text for sequences established by other workers. The centromere is located near *rg* in I, near *bal* in II and left of *thi-3* in VII.

BEADLE and FRASER 1935. Groups I and VI correspond to groups A and B of HOULAHAN, BEADLE and CALHOUN 1949.

These crosses were selected from a much larger number on the basis of several criteria. Two-point crosses have generally not been reported unless they involved markers for which adequate 3-point data were lacking. Crosses with low germination or poor allele ratios ordinarily were included only if they provide information that is not available from other crosses less likely to have been distorted by selective survival. The author is indebted to several persons for permission to use unpublished data, as indicated in footnotes to the tables.

Linkage of amycelial to sex was first established by DR. K. C. ATWOOD. Linkage of osmotic in group I was first shown by MRS. MARY R. EMERSON, and of *un* (STL6) by DR. P. ST. LAWRENCE. Location of *arg-5* in II was suggested by DR. R. W. BARRATT on the basis of its linkage to *al*(4736T) (SRB 1946). Linkage of *arg-11* in group VII was discovered by DR. D. NEWMEYER. *nic-3* (Y31881) and *me-7*(4894) were first shown to be linked in VII by MRS. M. K. ALLEN; their location was aided by unpublished data of MRS. MIRIAM BONNER.

Evidence for allelism or close linkage was obtained from intercrosses between mutants that appeared to be recurrences. The numbers of mutant progeny among which no wild type recombinants occurred were: B187 × *rg*(B53) : 68. STL4 × *lys-4*(15069) : 556. B74 × $cr^L$ : 15. B122 × $cr^L$ : 16. B123 × $cr^L$ : 181. B74 × *cr* (B123) : 14. B122 × *cr*(B123) : 13. B180 × *cr*(B123) : 115. B102 × *aur*(34508) : 53. B102 × *al-2*(15300) : 23. JH216 × *al-2*(15300) : 23 (plus one wild type). JH9698 × *al-2*(15300) : 19. B102 × $al^s$ : 22 (probable *al-2* allele, SANSOME *et al.* 1945). $al^c$ × *al*(B102) : 24. Y602 × *al*(B102) : 27. Y2170 × *al*(B102) : 23. Y2171 × *al*(B102) : 27. $al^M$ × *hs*(51504) : 76 (data of E. G. BARRY). B135 × *os* (E11200) : 124 (including data of P. ST. LAWRENCE). M16 × *os*(E11200) : 113 (data of ST. LAWRENCE). M155–5 × $fl^L$ (LINDEGREN's) : 136. P346 × $fl^L$ : 124. P605 × $fl^L$ : 124. $fl^P$ × $fl^L$ : 168. B148 × *col-4*(70007) : 138. B12 × *bis*(B6) : 189. B30 × *bis*(B6) : 123.

*Relation to the data of other investigators:* The maps presented in Figure 1 are based solely on the data from the present paper, and therefore include only the markers used in our crosses. For the convenience of the reader, all known markers in groups I and VII are listed in Tables 4 and 5, together with references to the work of other investigators that bears on their status as markers.

A number of gene sequences that were not included in the 1954 compilation of BARRATT *et al.* have been established or confirmed by other workers. In linkage Group I these are: *leu-3 sex phen* centromere *ad-3B cr* (BARRATT and OGATA 1954); *sex hist* (K12) 0.7 *hist* (K26) *arg-3 al-2* (MATHIESON and CATCHESIDE 1955, most likely order); *sex arg-1 arg-3* (NEWMEYER 1957, most likely order); *sex* 4.4 *ad-5* 2.2 centromere 6.1 *vis* (3717) (HOWE 1956); centromere 0.5 *hist-2* 2.0 *ad-3A* 0.1 *ad-3B* 3.0 *nic-2* (DE SERRES 1957); *ad-3 T al-2* (HOROWITZ and FLING 1956); and *al-2 lys-3 nic-1 os* (ST. LAWRENCE 1956). In linkage Group VII: centromere *sfo thi-3 nt* (REISNER, BARRATT and NEWMEYER 1953); and *bn arg-10 nt* (NEWMEYER 1957). The data in the present paper are consistent with these results.

There remain a number of uncertainties regarding gene order, such as the sequence of *amyc* and *un*(55701t) with respect to mating-type; *ad-5* and *ti* with respect to *arg-1; st* with respect to *cr*; and *bal* and *arg-5* with respect to centromere and *pe*. (The seriation shown for *ti* and *arg-1* depends on a single isolate.)

Markers and sequences in linkage groups III, IV, and V are considered in the three accompanying papers of this series. Our contributions to groups II and VI have been insufficient to justify separate tabulations for them. Information not included in BARRATT *et al.* 1954 will be found in the following publications:

STADLER 1956a, b; TOWE 1958 (Group VI map; *asco*); PITTENGER 1954 (*cys-1*, *cys-2*, VI); CASE 1957, 1958; CASE and GILES 1958a, b (*ylo ad-1 pan-2 tryp-2*, VI); HOULAHAN, BEADLE and CALHOUN 1949 (*phen*(38602), VI).

## DISCUSSION

New markers and more reliable maps are obviously useful as tools for cytogenetic work. The data reported here and in the accompanying papers on groups III, IV and V may also be of interest for the information they provide on interference, the distribution of loci within the chromosome complement, and the variability of crossing over. Results from all four papers in the series will be discussed together here in relation to these subjects.

*Interference:* The present results from random segregants indicate that Neurospora does not differ essentially from higher organisms such as Drosophila with respect to chiasma interference. A close similarity to Drosophila is also apparent in tetrad data (PERKINS 1958). On the other hand, Neurospora does appear to differ from the homothallic ascomycete *Aspergillus nidulans*, where no evidence of positive chiasma interference has been obtained either with random isolates (KÄFER 1958) or with tetrads (STRICKLAND 1958).

An excess of double crossovers ("negative interference") has been reported in Aspergillus for very short intervals where selective techniques of analysis are normally employed (PRITCHARD 1958). A similar excess of double crossovers was also reported in nonselective experiments with longer intervals by CALEF (1957), who pointed out, however, that the numbers obtained nonselectively were too small to be conclusive. Computation of fiducial limits for double crossovers shows that the numerical coincidence value of 3.0, obtained by CALEF in

TABLE 4

*Loci of linkage group I*

| Symbol and name* | Standard mutant | References† |
|---|---|---|
| *a* : *sex* or mating type | .... | B |
| *ad-3A* : adenine-3A | 38701 | B, DE SERRES 1956, 1958; GILES *et al.*, 1957; DE SERRES *et al.*, 1958 |
| *ad-3B* : adenine-3B | 35203 | B, DE SERRES 1956, 1958; GILES *et al.*, 1957; BARRATT and OGATA 1954; DE SERRES *et al.*, 1958 |
| *ad-5* : adenine-5 | 71104 | B, HOWE 1956 |
| *al-1* : albino-1 (see aurescent) | | |
| *al-2* : albino-2 | 15300 | B, PITTENGER 1954; ST. LAWRENCE 1956 |
| *amyc* : amycelial | K422 | ATWOOD 1949 |
| *arg-1* : arginine-1 | 46004 | B, NEWMEYER 1957 |
| *arg-3* : arginine-3 | 30300 | B, NEWMEYER 1957; MATHIESON *et al.*, 1955 |

| | | |
|---|---|---|
| *arg-6* : arginine-6 | 29997 | B |
| *aur* : aurescent | 34508 | B, PITTENGER 1954 |
| *bl* : black colonial | C113 | SCHAEFFER 1953 (Lost) |
| *can* : canavanine resistant | 46003R | B |
| *cr* : crisp | $cr^{L}$‡ | B, BARRATT and OGATA 1954; MALING 1959a |
| *cyt*(C115) : cytochrome | C115 | MITCHELL *et al.*, 1953 |
| *dir* : dirty | ‡ | B (Lost? Possibly = *os*) |
| *fr* : frost | B110 | |
| *gap* : gap | ‡ | B (Lost?) |
| *hist-2* : histidine-2 | C94 | B, DE SERRES 1956; GILES *et al.*, 1957 |
| *hist-3* : histidine-3 | C140 | B |
| *hist*(K12) : histidine | K12 | MATHIESON *et al.*, 1955 |
| *hist*(K26) : histidine | K26 | MATHIESON *et al.*, 1955 |
| *hs* : homoserine | 51504 | B |
| *leu*-3 : leucine-3 | 47313 | B, BARRATT and OGATA 1954 |
| *lys-3* : lysine-3 | 4545 | B |
| *lys*(28815) | | B (Allelic *lys-3*(4545), ST. LAWRENCE, personal communication) |
| *lys-4* : lysine-4 | 15069 | B |
| *mac* : methionine-adenine-cystine | 65108 | DUBES 1953 (Possible allele *me-6*) |
| *me-6* : methionine-6 | 35809 | B (Assigned locus number on basis of cross 40, Table 2, showing nonallelism with *me-1*) |
| *mt* : mating type (see *a*) | | |
| *m-1(vis)* : modifier-1 of *vis*(3717) | .... | HOWE 1956 |
| *m-2(vis)* : modifier-2 of *vis*(3717) | .... | HOWE 1956 |
| *nd* : natural death | .... | B (Lost?) |
| *nic-1* : nicotinic-1 (= *q*) | 3416 | B, ST. LAWRENCE 1956 |
| *nic-2* : nicotinic-2 | 43002 | B, DE SERRES 1956; GILES *et al.*, 1957 |
| *nit* : nitrate nonutilizer | 34547 | B |
| *n-nit* : nitrate nonutilizer (see *nit*) | | |
| *os* : osmotic | E11200 | EMERSON *et al.*, 1958; ST. LAWRENCE 1956 |
| *pa* : pale | ‡ | B (Lost?) |
| *phen* : phenylalanine | H6196 | BARRATT and OGATA 1954 |
| *q* : quinolinic acid (see *nic-1*) | | |
| *rg* : ragged | B53 | MALING 1959a |
| *sex* : sex (see *a*) | | |
| *slo* : slow | B149 | (No allelism test with *cyt*(C115) |
| *sn* : snowflake | C136 | MITCHELL 1958 |
| *so* : soft | B230s | |
| *st* : sticky | B128 | |
| *su-1-me* : suppressor-1 of methionine | R1 | B, FISCHER 1957 |
| *suc* : succinic | 35402 | B |
| *T* : tyrosinase thermostability | .... | HOROWITZ *et al.*, 1953, 1956 |
| *thi-1* : thiamine-1 | 56501 | B, EBERHART 1956 |
| *ti* : tiny | B233t | |
| *un*(46403): unknown requirement | | B (Probably *suc* DUBES 1953) |
| *un*(STL6) : unknown requirement | STL6 | |
| *un*(44409) : unknown requirement | 44409t | B |
| *un*(55701) : unknown requirement | 55701t | B |
| *vis*(3717) : visible | 3717 | B, HOWE 1956 |

* Mutants believed to be lost, mutants of doubtful value as markers, and mutants that are probably alleles at already established loci are indented.

† B: Documented in BARRATT *et al.*, 1954 compilation. Additional references are cited only if they provide new information on linkage, scoring, or gene structure.

‡ The standard mutant is LINDEGREN's. (No isolation number.)

TABLE 5

*Loci of linkage group VII*

| Symbol and name | Standard mutant | References* |
|---|---|---|
| *arg-10* : arginine-10 | B317 | B, NEWMEYER 1957 |
| *arg-11* : arginine-11 | 30820 | SRB 1950 |
| *bn* : button | B40 | NEWMEYER 1957 |
| *me-7* : methionine-7 | 4894 | BUSS 1944; M. ALLEN unpub. |
| *nic-3* : nicotinic-3 | Y31881 | BONNER *et al.*, 1949; M. ALLEN unpub. |
| *nt* : nicotinic-tryptophan | 65001 | B, REISNER *et al.*, 1953; NEWMEYER 1957 |
| *sfo* : sulfonamide requiring | E15172 | B, REISNER *et al.*, 1953 |
| *sk* : skin | B106 | |
| *thi-3* : thiamine-3 | 18558 | B, REISNER *et al.*, 1953; EBERHART 1956 |

* B: Documented in BARRATT *et al.*, 1954 compilation. Additional references are cited only if they provide new information on linkage, scoring, or gene structure.

his most reliable nonselective analysis (intervals 1 and 2, Table 1, 1957), is not significantly greater than a minimum coincidence of 0.4.

The present data from Neurospora, which were obtained nonselectively, provide no indication of negative interference. On the contrary, interference is typically positive. Coincidence values are given in the tables wherever one or more double crossovers occurred in adjacent intervals. In a few crosses (e.g., 1, 4, 5, 8 in Table 2) the numerical coincidence value exceeds one. MOLINA's (1942) and STEVENS' (1942) tables were used in all such cases to test the significance of the excess doubles observed, and to compute the minimum coincidence values from which observed numbers of doubles were deviates at the 2½ percent one-sided level (equivalent to a conventional five percent level). In no case is the observed excess of doubles statistically significant. Only six double crossovers were observed within intervals less than 15 units long, in groups IV and V. Although coincidence exceeds one in these cases, the double crossovers observed do not constitute statistically significant evidence of negative interference.

The data presented here give no information as to whether exchanges might occur as doubles or clusters within very short regions (see FREESE 1957; PRITCHARD 1958) but such a model seems unlikely from a consideration of various tetrad data (WEINSTEIN 1957).

*Distribution of loci:* A total of approximately 125 loci have now been mapped in nine of the 14 chromosome arms of Neurospora, but only in Groups I and VI are markers known that are located at appreciable distances from the centromere in both chromosome arms. The remaining five arms are still effectively devoid of markers. Such a distribution is unexpected on the basis of cytological observations, which indicate that all seven centromeres are definitely nonterminal in location (MCCLINTOCK 1945; SINGLETON 1953).

Group I, the mating type group, now contains about 40 known loci. It has been extended in both directions by the mapping of frost on the left and of soft and osmotic on the right. This reinforces its status as the best marked group of the complement. Group I also exceeds any other group in map length (over 125

units), even though cytologically it is located in the second shortest chromosome of the complement, chromosome 6 (ST. LAWRENCE 1952).

Genes in linkage group II (fluffy, *arom*, etc.) are located in the long arm of chromosome 1, which is cytologically the longest chromosome (ST. LAWRENCE 1952; MCCLINTOCK 1945). Known genes in linkage group IV (*pdx-1*, *pan-1*, etc.) are probably all located in the short (nucleolus organizer) arm of chromosome 2, which is the second longest chromosome (ST. LAWRENCE 1952). The short arm of chromosome 1 and the long arm of chromosome 2, although they are devoid of genetic markers, are nevertheless each longer than the best mapped chromosome, 6, in its entirety (see SINGLETON 1953).

Groups III, V, and VII have not yet been assigned to specific chromosomes, but regardless of whether chromosomes 3, 4, 5, or 7 are involved, the same question is posed. Why have loci not been identified in both arms?

It may be that the strikingly uneven distribution of genes is merely a chance result that reflects the location of markers that happen to have been used for testing linkage. The long-standing availability of mating-type and albino for linkage tests in I has no doubt contributed substantially to locating markers throughout this linkage group. In other groups, testing has frequently been carried out with markers far removed from the centromere, e.g., *fl* in II, *pan-1* in IV, *inos* in V, and *nt* in VII. Linkage of genes in the opposite arms might have gone undetected in such tests.

It is also possible that the "empty" arms may be genetically inert, or that mutations occurring in them may be of such a nature that they are not recovered as typical markers. It should be possible to obtain evidence distinguishing the main alternatives. Suitable markers close to the centromere in each group are now available for linkage tests that should enable any gene loci in the five unmapped arms to be identified. Table 6 lists the genes that seem to be most suitably located for extending the various linkage groups beyond their present limits.

TABLE 6

*Useful markers for the centromere and for extremes of each linkage group*

| Centromere markers | I | II | III | IV | V | VI | VII |
|---|---|---|---|---|---|---|---|
| Visible | *cr* | *bal* (B56) | *sc* (5801) | (*col-4*) (70007) | (*sp*) (B132) | *ylo* (Y30539y) | *bn* (B40) |
| Nutritional | *hist-2* (C94) | *arg-5* (27947) | *thi-4* (85902) | *pyr-1* (H263) | *lys-1* (33943) | *ad-1* (3254) | *me-7* (4894) |

| Distal markers | I-L | I-R | II-R | III-R | IV-R | V-R | VI-L | VI-R | VII-R |
|---|---|---|---|---|---|---|---|---|---|
| Visible | *fr* (B110) | *os* (E11200) | *fl* | *vel* (B18) | *mat* (B57) | *pl* (B118) | *del* (B137) | — | *sk* (B106) |
| Nutritional | (*leu-3*) (47313) | (*nic-1*) (3416) | *tryp-3* (C83) | *tyr* (Y6994) | *pyr-2* (38502) | *asp* (S1007) | *asco* (37402) | *tryp-2* (75001) | (*nt*) (65001) |

Markers with symbols in parentheses are located ten or more units from the centromere or from the most distal marker *rg*(B53) is closer to centromere than *cr*, and *so*(B230s) and *cyt*(C117) are more distal than *os* and *asco* respectively, but *cr*, *os*, and *asco* are probably preferable for technical reasons.

*Recurrences:* Eight previously unmapped albino mutants of various origins are reported in Table 1. These are all apparently located within the same region of group I as the eight albino mutants previously mapped by HUNGATE (1945). Visible mutants have also been recovered recurrently at a number of other loci: crisp (4 recurrences), fluffy (4), biscuit (3), osmotic (2), ragged (2), ropy-1 (2), skin (2), and colonial-4 (1).

*Variability of crossing over:* Striking differences in recombination frequencies were observed for identical intervals in a number of the crosses reported here, in spite of the fact that markers had been more or less inbred to standard strains in an effort to decrease heterogeneity. The differences in crossing over between crosses of different parentage are great enough to have led to spurious conclusions regarding gene order if sequences had been determined only on the basis of two-point data combined from different crosses. This source of error can be avoided by using multiple point crosses, which enable order to be decided on the basis of data from a single cross. Three-point data have been used wherever possible to indicate gene order in the present study, and they have done so unambiguously in most cases, even though the number of segregants has been small.

A detailed statistical treatment of the crossing over variations observed here does not seem justified in view of the fact that the experiments were not designed to obtain information on variability, and no effort was made to analyze large numbers of progeny from crosses that showed unusually high or low recombination frequencies. Consequently, sampling error often cannot be eliminated as a possible explanation. Selection may well be responsible for several deviant recombination values, in crosses where low germination or poor allele ratios were observed. (This is probably true, for example, of crosses 21, Table 2; 12, Table 3; and 24, group V.) The remaining variations in crossing over, that cannot be explained in these ways, are probably genetic rather than environmental in origin. Crosses were carried out under carefully controlled standard conditions, and similar recombination values have been obtained consistently when crosses between the same two parents were repeated. Reproducibility of results has also been observed by MALING (1959a), in crosses repeated on a large scale.

STADLER (1956a, c) and TOWE (1958) have used tetrads to obtain extensive information regarding the genetic control of crossing over in Neurospora. Exchange frequencies between their group VI markers remained constant in repeated crosses between the same two parents, but crossing over varied widely in crosses of different parentage. In their experience, crossing over generally increased (and never decreased) in successive backcross generations.

In the present work, group I markers were most inbred, group IV probably least, before being used to obtain linkage data. The group I crossover values reported here are consistently greater than those collected from heterogeneous sources by BARRATT *et al.* (1954). On the other hand, the group IV values reported here by MALING are somewhat smaller than those in BARRATT *et al.*, and only about one half as great as those of MITCHELL and MITCHELL 1954.

It is not surprising that crossover frequencies are heterogeneous in Neurospora,

where mutants were originally obtained starting with a variety of different wild type strains, where exposure to X-rays and other mutagens has often been high, and where stocks have commonly been maintained as vegetative clones for long periods of time through repeated transfers. With respect to recombination variability, as with interference, Neurospora resembles Drosophila and maize more nearly than it resembles *Aspergillus nidulans*. The striking homogeneity of crossover values in Aspergillus may be related to the fact that all genetic work has employed strains tracing back to a single nucleus (see KÄFER 1958).

## SUMMARY

Fifty-three previously unmapped mutants of *Neurospora crassa* are described, of which at least 25 are at new loci.

Data on random segregants obtained by nonselective methods from 75 multiple-point crosses have placed 14 of the previously unmapped loci in linkage groups I, II, VI, and VII, and have clarified the sequence of previously known genes. These results, taken together with new data in a series of three accompanying papers on linkage groups III, IV, and V, bring the total number of mapped loci in Neurospora to over 130, and the number of loci in group I to at least 40. All of the mapped loci fall in only nine out of the 14 chromosome arms. (A few genes near the centromeres are possible exceptions.) Coincidence values range from zero to not significantly greater than one for neighboring regions of increasing length. Gene sequences have been established by 3-point tests rather than by combining data from 2-point crosses, in order to avoid errors due to variations in crossing over frequency.

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# LINKAGE DATA FOR GROUP III MARKERS IN NEUROSPORA[1]

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THIS paper presents data from crosses with markers in linkage group III of *Neurospora crassa* (group C of HOULAHAN, BEADLE, and CALHOUN 1949). Participation of the second author in this study was prompted by the discovery by DR. P. ST. LAWRENCE of a chromosomal rearrangement involving group III, and the desire for information on markers and linkage relations that might be useful for investigating it. Three new loci have been placed in the group, and additional information has been obtained on the relations of ten previously mapped genes. All the gene loci now known to be linked in group III are listed alphabetically in Table 1.

## MATERIALS AND METHODS

General experimental materials and methods were as described in PERKINS 1959. The new morphological mutants B54 (*com* : compact), B20 (*ro-2* : ropy-2) and B18 (*vel* : velvet) originated in experiments of DR. V. W. WOODWARD (see Table 1 of PERKINS 1959).

TABLE 1

*Loci of linkage group III*

| Symbol and name | Standard mutant | References* |
|---|---|---|
| *ad-2* : adenine-2 | 70004t | B, PITTENGER 1954 |
| *ad-4* : adenine-4 | 44206t | B, GILES *et al.*, 1957; WOODWARD *et al.*, 1958 |
| *com* : compact | B54 | PERKINS 1959 |
| *leu-1* : leucine-1 | 33757 | B |
| *prol-1* : proline-1 | 21863 | B |
| *ro-2* : ropy-2 | B20 | PERKINS 1959 |
| *sc* : scumbo | 5801 | B |
| *ser* : serine | H605 | B |
| *thi-2* : thiamine-2 | 9185 | B, EBERHART 1956 |
| *thi-4* : thiamine-4 | 85902 | B, EBERHART 1956; EBERHART *et al.*, 1959 |
| *thi-lo* : low-thiamine† | .... | EBERHART 1956; EBERHART *et al.*, 1959 |
| *tryp-1* : tryptophan-1 | 10575 | B, PITTENGER 1954 |
| *tyr* : tyrosine | Y6994 | B |
| *vel* : velvet | B18 | PERKINS 1959 |

* B: Documented in BARRATT *et al.*, 1954 compilation. Additional references are cited only if they provide new information on linkage, scoring, or gene structure.
† Probable allele of *thi-4*.

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TABLE 2

*Crosses with group III markers*

| Zygote genotype and recombination percent | Parental combinations | | Recombinations: Singles region 1 | | Singles region 2 | | Doubles regions 1 and 2 | | Total and percent germination | Marker isolation numbers |
|---|---|---|---|---|---|---|---|---|---|---|
| + *b* *c* / *a* + + | +*bc* | *a*++ | +++ | *abc* | +*b*+ | *a*+*c* | ++*c* | *ab*+ | ... | (a) (b) (c) |
| + *thi-4* / *sc* + 0.8 | 65 | 61 | 1 | 0 | .. | .. | .. | .. | 127<br>83% | 5801<br>85902 |
| + *prol-1* *tryp-1* / *sc* + + 7.2 29.9 | 40 | 22 | 5 | 1 | 11 | 17 | 1 | 0<br>C=0.5 | 97<br>97% | 5801<br>21863<br>10575 |
| + *prol-1* *com* / *thi-4* + + 2.6 7.7 | .. | 35 | 1 | .. | 3 | .. | .. | 0 | 39<br>(*com*⁺ only)<br>73% | 85902<br>21863<br>B54 |
| + *com* *leu-1* / *thi-4* + + 15.4 3.3 | 34 | 40 | 13 | 1 | 2 | 1 | 0 | 0 | 91*<br>91% | 85902<br>B54<br>33757 |
| + *tryp-1* + / *thi-4* + *ro-2* 21.7 14.5 | 23 | 23 | 9 | 4 | 5 | 3 | 1 | 1<br>C=0.9 | 69<br>86% | 85902<br>10575<br>B20 |
| + *com* *leu-1* / *ser* + + 2.4 2.4 | 41 | 39 | 2 | 0 | 2 | 0 | 0 | 0 | 84*<br>84% | H605<br>B54<br>33757 |
| + *com* + / *prol-1* + *tryp-1* 6.1 6.1 | .. | 29 | 2 | .. | .. | 2 | 0 | .. | 33<br>(*com*⁺ only)<br>63% | 21863<br>B54<br>10575 |
| + + *vel* / *prol-1* *tryp-1* + 23.8 33.3 | 12 | 9 | 3 | 4 | 4 | 7 | 1 | 2<br>C=0.9 | 42<br>72% | 21863<br>10575<br>B18 |
| + *ad-4* + / *com* + *leu-1* 0 2.3 | 95 | 74 | 0 | 0 | 4 | 0 | 0 | 0 | 173*<br>84% | B54<br>44206t<br>33757 |
| + *ad-4* *tryp-1* / *com* + + 5.4 15.5 | 61 | 72 | 5 | 4 | 19 | 7 | 0 | 0 | 168*<br>84% | B54<br>44206t<br>10575 |
| + *leu-1* / *com* + 4.6 | 114 | 92 | 8 | 2 | .. | .. | .. | .. | 216*<br>83% | B54<br>33757 |
| + + *thi-2* / *com* *leu-1* + 3.4 3.8 | 90 | 104 | 1 | 6 | 6 | 2 | 0 | 0 | 209*<br>82% | B54<br>33757<br>9185 |
| + *tryp-1* / *ad-4* + 9.0 | 83 | 68 | 12 | 3 | .. | .. | .. | .. | 166*<br>87% | 44206t<br>10575 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| + + *ad-2*<br>*tryp-1* *thi-2* +<br>0 0.6 | 81 | 96 | 0 | 0 | 0 | 1 | 0 | 0 | 178*<br>89% | 10575<br>9185<br>27663 |
| + *thi-2* *vel*<br>*tryp-1* + +<br>1.2 20.7 | 64 | .. | .. | 1 | .. | 17 | 0 | .. | 82<br>(*vel*-only)<br>90% | 10575<br>9185<br>B18 |
| + *ad-2* +<br>*tryp-1* + *vel*<br>0.5 13.6 | 75 | 101 | 0 | 1 | 11 | 17 | 0 | 0 | 205*<br>82% | 10575<br>27663<br>B18 |
| + + *tyr*<br>*tryp-1* *ro-2* +<br>2.3 22.6 | 56 | 44 | 1 | 2 | 14 | 16 | 0 | 0 | 133<br>93% | 10575<br>B20<br>Y6994 |
| + + +<br>*tryp-1* *ro-2* *tyr*<br>8.0 15.9 | 52 | 53 | 7 | 4 | 12 | 10 | 0 | 0 | 138<br>55% | 10575<br>B20<br>Y6994 |
| + + *tyr*<br>*tryp-1* *vel* +<br>17.4 3.3 | 71 | 75 | 15 | 17 | 4 | 2 | 0 | 0 | 184*<br>92% | 10575<br>B18<br>Y6994 |
| + *tyr*<br>*vel* +<br>5.1 | 70 | 97 | 6 | 3 | .. | .. | .. | .. | 176*<br>88% | Y6994<br>B18 |

Numbers of progeny are given in the body of the table. Progeny genotypes are not designated explicitly, but the genotype of each class can be determined from the order of presentation. The left-hand number of each pair of complementary classes represents the genotype that contains the plus allele of the leftmost marker. Classes of segregants where dashes replace numbers were not scored or recorded. Regions are numbered from left to right, and isolation numbers in the last column are listed in the same order. Crosses are tabulated in a sequence corresponding to the position of their leftmost markers in the linkage group. Where gene order is uncertain, one arbitrarily chosen order has been used consistently. C=coincidence.

* Data of the second author.

Segregants from crosses involving *tryp-1* were grown on supplemented minimal medium containing 10 μg/ml indole and scored at three days (34°) by fluorescence under ultraviolet light. (A 4-watt Blak-Ray lamp is inexpensive and effective. Ultra-violet Products, Inc., San Gabriel, Calif.: Model X4, wavelength 3660.)

## EXPERIMENTAL RESULTS

Data from two- and three-point crosses are presented in Table 2, and the results are summarized in the form of a map in Figure 1. These data provide new information on the map relations of several genes. *com* is probably located just proximal to *ad-4*. *ro-2* is between *tryp-1* and *tyr*, and nearest to the former. *vel* is located a short distance from the terminal marker, *tyr*.

Extensive information on sequences in group III was first obtained by HUNGATE (1946), who established the order centromere *sc ser prol-1 leu-1 tryp-1* (or *thi-2*). Subsequent work established the sequences centromere *tryp-1 tyr* (BARRATT, NEWMEYER, PERKINS and GARNJOBST 1954) and *prol-1 ad-4 leu-1* (GILES, PARTRIDGE and NELSON 1957). The data reported here are consistent with the results of these authors.

A number of Group III sequences are still in doubt, notably those of closely linked genes. The seriation shown for *thi-2* and *ad-2* with respect to *tryp-1*

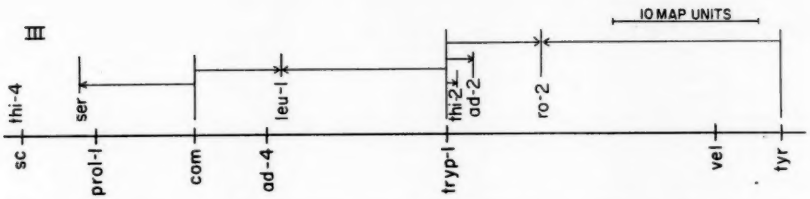


FIGURE 1.—Partial map of group III, summarizing the data from Table 2. The sequence of loci written below the heavy line is based directly on 3-point data for all the genes involved. Above the heavy line, only the relationships indicated by arrows are based on 3-point data. Two opposing arrows indicate that the marker was situated medially in the 3-point cross; each unopposed arrow originates from a medial marker and leads to a marker that was not medial. Interval lengths are imprecise owing to the variability of crossing over. The map is based solely on data from the present paper, except that the position shown for genes above the line may reflect the results of other workers in cases where our own data would require order to be decided arbitrarily or to be based only on quantitative 2-point data from different crosses. See text for sequences established by other workers. The centromere is located just left of *sc*.

depends in each case on a single isolate. It would be desirable to have further 3-point data clarifying the sequence of these loci, and of *thi-4* with respect to *sc* and the centromere.

scumbo, which is near the centromere, was used in tests for linkage in Group III. This should be equally effective in detecting linkage of new markers in either arm of the chromosome. However, the three new markers reported here all proved to be located in the same arm with the ten previously known loci.

A general discussion of these results, in conjunction with comparable data from the other linkage groups, will be found in PERKINS 1959, where such aspects as interference and the variability of crossing over are considered.

## SUMMARY

Linkage data on 13 gene loci in linkage group III of *Neurospora crassa* have been obtained by nonselective techniques using random segregants, mostly from 3-point crosses. Three of the loci have not been known previously. All mapped genes are located in the same chromosome arm (with the possible exception of one which is close to the centromere). Interference is apparently orthodox.

## ACKNOWLEDGMENTS

We are grateful to DR. E. L. TATUM, MRS. MARY B. MITCHELL, and DR. V. W. WOODWARD for providing the mutant strains used in this study, and to MRS. MABYN MARTIN for technical assistance.

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TABLE 1

*Loci of linkage group IV*

| Symbol and name | Standard mutant | References* |
|---|---|---|
| *ad-6* : adenine-6 | 28610 | B, M |
| *arg-2* : arginine-2 | 33442p | B, M, REISSIG 1958 |
| *chol-1* : choline-1 | 34486 | B |
| *col-1* : colonial-1 | Y8743c | B |
| *col-4* : colonial-4 | 70007 | B, M |
| *cot* : colonial-temperature sensitive | C102 | M |
| *dn* : dingy | 38502 | M |
| *hist-4* : histidine-4 | C141 | B, M |
| *hist*(K34) : histidine† | K34 | MATHIESON *et al.*, 1955 |
| *leu-2* : leucine-2† | 8839 | REGNERY 1947 |
| *leu*(37501) : leucine | 37501 | REGNERY 1947 |
| *mat* : mat | B57 | PERKINS 1959 |
| *me-2* : methionine-2 | H98 | B, FLING *et al.*, 1957 |
| *me-5* : methionine-5 | 9666 | B, FLING *et al.*, 1957 |
| *pan-1* : pantothenic-1 | 5531 | B, M |
| *pdx-1* : pyridoxine-1 | 35405 | B, M, MITCHELL 1955a, b, 1956; PITTENGER 1954 |
| *pdx-2* : pyridoxine-2 | 44204p | B, PITTENGER 1954 |
| *pyr-1* : pyrimidine-1 | H263 | B, M, SUYAMA *et al.*, 1957 |
| *pyr-2* : pyrimidine-2 | 38502 | B, M |
| *pyr-3* : pyrimidine-3 | 37815t | B, M, MITCHELL *et al.*, 1956; SUYAMA *et al.*, 1957; REISSIG 1959 |
| *pyr-5* : pyrimidine-5 | KS12 | WOODWARD *et al.*, 1958 |
| *pt* : phenylalanine-tryrosine | S4342 | COLBURN 1958 |
| *rib-2* : riboflavin-2 | Y30539r | B, GARNJOBST *et al.*, 1956 |
| *ro-1* : ropy-1 | B4 | PERKINS 1959 |
| *tryp-4* : tryptophan-4 | Y2198 | B |

* B: Documented in BARRATT *et al.*, 1954 compilation. M: Data and map in MITCHELL *et al.*, 1954. Additional references are cited only if they provide new information on linkage, scoring, or gene structure.

† See text.

*col-4 arg-2 pyr-3—ad-6 pan-1 cot hist-4—pyr-2 dn.* (The genes were grouped in three rather tightly linked clusters, set off here by dashes.) Other sequences reported since 1954 are: *me-2 cot me-5* (FLING, HOROWITZ and REINKING 1957); *pdx-1 rib-2 pyr-3 chol-1* (GARNJOBST and TATUM 1956); centromere *pyr-3 pyr-5* (WOODWARD, SUYAMA and MUNKRES 1958); *pdx-1* 1.9 *col-4* 1.0 *pt* (COLBURN 1958).

The data reported here are qualitatively consistent with sequences arrived at by these authors. Recombination frequencies have generally been lower in our crosses than in those of MITCHELL and MITCHELL 1954, but this would not affect decisions on gene order, which are based on 3-point data.

A number of group IV sequences are not yet established with certainty. The seriation shown for *cot* and *chol-1* with respect to *ad-6*, and of *tryp-4* and *leu* (37501), depends in each case on a single isolate. Further 3-point crosses are needed to clarify the positions of *me-2*, *tryp-4*, *leu*(37501), *chol-1*, *me-5*, and *hist-4*. Some other sequences of closely linked genes may also require confirmation (MITCHELL 1958).

*Status of new mutants:* Mutant B15 is phenotypically indistinguishable from *ro-1* (B4). Crosses between the two are sterile, but both of them are closely linked

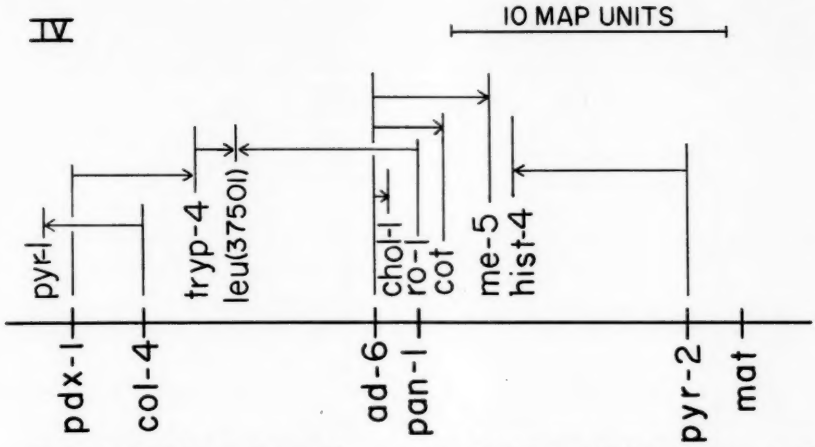


FIGURE 1.—Partial map of group IV, summarizing the data from Table 2. The sequence of loci written below the heavy line is based directly on 3-point data for all the genes involved. Above the heavy line, only the relationships indicated by arrows are based on 3-point data. Each arrow originates from the medial marker in the 3-point cross, and leads to a marker that was non-medial. Interval lengths are imprecise owing to the variability of crossing over. The map is based solely on data from the present paper, except that the position shown for genes above the line may reflect the results of other workers in cases where our own data would require order to be decided arbitrarily or to be based only on quantitative 2-point data from different crosses. See text for sequences established by other workers. The centromere is located to the left of *pyr-1* and *pdx-1*.

to *pan-1* (crosses 17 and 18). B15 and B4 are therefore tentatively designated as alleles. Mutant B148 is phenotypically indistinguishable from *col-4* (70007), and no wild types occurred among 138 segregants from an intercross (PERKINS 1959). B148 and 70007 are therefore considered to be alleles.

*leu-2* (8839) and *leu*(37501) were shown by DR. D. C. REGNERY to be located in group IV, on the basis of linkage to *col-1*. The two mutants are phenotypically different (REGNERY 1947); genetic evidence of allelism between 8839 and 37501 is ambiguous because 8839 is unstable and reverts frequently to wild type. The author is indebted to DR. REGNERY for making available this unpublished information regarding linkage and allelism.

MATHIESON and CATCHESIDE (1955) reported a new histidine mutant, K34, which appeared to be located about 33 units distal to *pan-1*. In our hands, K34 has appeared to segregate independently of both *pan-1* and *pyr-2* (crosses 3 and 22). Isolates of K34 were used that had been obtained both from DR. BRUCE AMES and from DR. CATCHESIDE.

General aspects of the results reported in the present paper have been discussed by PERKINS (1959) in conjunction with comparable data from other linkage groups. Among the topics considered are interference, variability of crossing over, and the distribution of loci in the chromosomes.

TABLE 2

*Crosses with group IV markers*

| Zygote genotype and recombination percent | Parental combinations | | Recombinations: Singles region 1 | | Singles region 2 | | Doubles regions 1 and 2 | | Total and percent germination | Marker isolation numbers |
|---|---|---|---|---|---|---|---|---|---|---|
| + *b* *c* / *a* + + | +*bc* | *a*++ | +++ | *abc* | +*b*+ | *a*+*c* | ++*c* | *ab*+ | ... | (a) (b) (c) |
| + *col-4* *pan-1* / *pyr-1* + + 2.3 8.3 | 150 | 164 | 5 | 2 | 12 | 16 | 0 | 1 C=1.5 (>0.04) | 350 87% | H263 B148 5531 |
| + *col-4* *pan-1* / *pdx-1* + + 5.1 15.2 | 31 | 32 | 0 | 4 | 8 | 4 | 0 | 0 | 79 79% | 37803 B148 5531 |
| + + + (*hist*) / *pdx-1* *col-4* *pan-1* (+) 4.1 8.1 | 37 | 28 | 1 | 2 | 4 | 2 | 0 | 0 | 74 77% | 37803 B148 5531 (K34) |
| + *col-4* *pan-1* / *pdx-1* + + 1.9 9.7 | 153 | 212 | 5 | 3 | 18 | 22 | 0 | 0 | 413 84% | 37803 B148 5531 |
| + (*arg-2*) *tryp-4* *ad-6* / *pdx-1* (+) + + 2.7 5.4 | 63 | 75 | 4* | 0 | 5 | 3 | 0 | 0 | 150 79% | 37803 (33442) Y2198 28610 |
| + *ad-6* *cot* / *pdx-1* + + 3.6 1.8 | 22 | 30 | 2 | 0 | 0 | 1 | 0 | 0 | 55 82% | 37803 28610 C102 |
| + *pan-1* *mat* / *pdx-1* + + 24.4 7.3 | 26 | 31 | 4 | 15 | 2 | 3 | 1 | 0 C=0.7 | 82 82% | 37803 5531 B57 |
| + (+) + *hist* / *pdx-1* (*col-4*) *pan-1* + 12.2 56.8 | 17 | 10 | 5 | 0 | 20 | 18 | 0 | 4 C=0.8 | 74 77% | 37803 (B148) 5531 K34 |
| + *ro-1* + / *pdx-1* + *mat* 19.7 19.7 | 39 | 41 | 13 | 5 | 5 | 13 | 5 | 1 C=1.3 (>0.47) | 122 70% | 37803 B4 B57 |
| + + *pyr-2* / *pdx-1* *pan-1* + 17.0 15.1 | 21 | 16 | 5 | 3 | 4 | 3 | 1 | 0 C=0.7 | 53 86% | 37803 5531 38502 |
| + *leu* + / *tryp-4* + *pan-1* 1.0 10.0 | 46 | 34 | 0 | 1 | 5 | 4 | 0 | 0 | 90[P] 90% | Y2198 37501 5531 |
| + *ad-6* + / *tryp-4* + *chol-1* 6.4 0.9 | 47 | 56 | 2 | 5 | 0 | 1 | 0 | 0 | 111 88% | Y2198 28610 34486 |
| + + *pan-1* / *tryp-4* *ad-6* + 4.2 0.7 | 70 | 67 | 2 | 4 | 1 | 0 | 0 | 0 | 144 91% | Y2198 28610 5531 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| + + *me-5*<br>*tryp-4* *ad-6* +<br>4.2 4.2 | 57 | 51 | 1 | 4 | 3 | 2 | 0 | 0 | 118<br>59% | Y2198<br>28610<br>9666 |
| + + *mat*<br>*leu* *pan-1* +<br>6.2 23.4 | 23 | 34 | 2 | 3 | 7 | 12 | 0 | 0 | 81[P]<br>81% | 37501<br>5531<br>B57 |
| + *pan-1* *mat*<br>*ad-6* + +<br>1.6 6.5 | 23 | 33 | 0 | 1 | 2 | 2 | 0 | 0 | 61<br>61% | 28610<br>5531<br>B57 |
| + *pan-1*<br>*ro-1* +<br>0 | .. | 35 | 0 | .. | .. | .. | .. | .. | 35[P]<br>(*pan*$^+$ only)<br>87% | B15<br>5531 |
| + *pan-1*<br>*ro-1* +<br>0 | 85 | 92 | 0 | 0 | .. | .. | .. | .. | 177<br>79% | B4<br>5531 |
| + *pan-1* *mat*<br>*ro-1* + +<br>0 21.6 | 81 | 89 | 0 | 0 | 25 | 22 | 0 | 0 | 217<br>87% | B4<br>5531<br>B57 |
| + *pyr-2* +<br>*pan-1* + *mat*<br>8.6 1.6 | 80 | 88 | 4 | 11 | 0 | 2 | 1 0<br>C=3.9<br>(>0.10) | | 186<br>93% | 5531<br>38502<br>B57 |
| + *pyr-2* +<br>*hist-4* + *mat*<br>10.9 3.1 | 55 | .. | .. | 7 | .. | 2 | 0 | .. | 64[a]<br>48% | C141<br>38502<br>B57 |
| + +<br>*pyr-2* *hist*<br>45.7 | 35 | 22 | 27 | 21 | .. | .. | .. | .. | 105<br>84% | 38502<br>K34 |

Numbers of progeny are given in the body of the table. Progeny genotypes are not designated explicitly, but the genotype of each class can be determined from the order of presentation. The left-hand number of each pair of complementary classes represents the genotype that contains the plus allele of the leftmost marker. Alleles shown in parentheses in the genotype column were not scored or recorded, nor were classes of segregants where dashes replace numbers. Regions are numbered from left to right, and isolation numbers in the last column are listed in the same order. Crosses are tabulated in a sequence corresponding to the position of their leftmost markers in the linkage group. Where gene order is uncertain, one arbitrarily chosen order has been used consistently. C=coincidence. When C exceeds 1, the minimum coincidence is also given from which the observed value does not deviate significantly (2.5 percent one-sided level).

* Three +*arg*++ and one ++++.
[a] Plus 27 *mat*$^-$ not scored.
[P] Data of D. PERKINS.


## SUMMARY

Linkage data have been obtained for 14 loci (including three that are new) in linkage group IV of *Neurospora crassa*, using random segregants obtained by nonselective methods, predominantly from 3-point crosses. All loci are in the same chromosome arm. Interference is apparently orthodox.



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# LINKAGE DATA FOR GROUP V MARKERS IN NEUROSPORA[1]

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THIS paper presents linkage data from crosses with markers in linkage group V of *Neurospora crassa* (Group E of HOULAHAN, BEADLE and CALHOUN 1949). Several new markers are reported, and the map relations of a number of other genes are clarified. Many of these data were obtained in the course of an investigation of interference (STRICKLAND unpublished). Certain of the new markers are in positions where they should be useful for bracketing regions of special interest (e.g., spray, which was first mapped in the present study, has already been employed in investigations of recombination at the complex *am* locus (PATEMAN 1958)). A total of at least 17 gene loci are now known to belong to group V. These are listed, with references, in Table 1.

TABLE 1

*Loci of linkage group V*

| Symbol and name* | Standard mutant | References† |
|---|---|---|
| *ad-7* : adenine-7 | 44411 | B, MITCHELL 1958 |
| *am* : amination deficient | 47305 | BARRATT unpublished; FINCHAM 1951, 1959; FINCHAM *et al.*, 1957a,b |
| *asp* : asparagine | S1007 | TANENBAUM *et al.*, 1954; FREESE 1957a; DUBES 1953 |
| *bis* : biscuit | B6 | PERKINS 1959 |
| *G* : Gulliver | .... | REISSIG 1958 |
| *hist-1* : histidine-1 | C84 | B, GILES 1956; FREESE 1957a,b |
| *i* : enhancer of *am* | .... | FINCHAM *et al.*, 1957b |
| *inos* : inositol | 37401 | B, GILES 1956; GILES *et al.*, 1953; FREESE 1957a; PITTENGER 1954; LESTER *et al.*, 1959 |
| *iv-1* : isoleucine-valine-1 | 16117 | B, GILES 1956; PITTENGER 1954 |
| *iv-2* : isoleucine-valine-2 | 39709 | B, PITTENGER 1954; FREESE 1957b |
| *lys-1* : lysine-1 | 33933 | B |
| *lys-2* : lysine-2 | 37101 | B, GOOD 1952 |
| *me-3* : methionine-3 | 36104 | B, FREESE 1957a |
| *pab-1* : para-aminobenzoic-1 | 1633 | B, GILES 1956; FREESE 1957a; DRAKE 1956 |
| *pab-2* : para-aminobenzoic-2 | H193 | B, DRAKE 1956 |
| *pab-3* : para-aminobenzoic-3 | 71301 | (Not a separate locus from *pab-2*. DRAKE 1956) |
| *pl* : plug | B118 | PERKINS 1959 |
| *sp* : spray | B132 | PATEMAN 1958; PERKINS 1959 |
| *val* : valine | 45201 | PERKINS 1959 |
| *wl* : woolly | B66 | PERKINS 1959 |

* Mutants of doubtful value as markers, and mutants that are probable alleles at already established loci are indented.
† B: Documented in BARRATT *et al.*, 1954 compilation. Additional references are cited only if they provide new information on linkage, scoring, or gene structure.

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## MATERIALS AND METHODS

Experimental materials and methods were as described by PERKINS (1959). The morphological mutants *bis* : biscuit (B6), *pl* : plug (B118), *sp* : spray (B132), and *wl* : woolly (B66) originated in experiments of DR. V. W. WOODWARD (see PERKINS 1959,Table 1). Scoring of *am* is cleaner at 25°C than at higher temperatures. Patterns of defective ascospores suggest that *wl* (B66) may be associated with a chromosomal aberration. An unlinked aberration may also have been present in some early crosses involving *asp*(S1007).

## EXPERIMENTAL RESULTS

Data from 2- and 3-point crosses are presented in Table 2, and from 4- and 5-point crosses in Table 3. The results are summarized in the form of a map in Figure 1.

*Gene order:* These data provide new information on a number of gene sequences: *sp* is located between *iv-1* and *am*, *bis* between *me-3* and *pab-2*, *me-3* between *pab-1* and *bis*, *val* at or near *iv-1*, *lys-2* rather far left of *inos*, and *ad-7* some distance right of *bis*. *asp*(S1007) and *pl* are close together far out in the right arm.

Since the 1954 compilation of BARRATT, NEWMEYER, PERKINS and GARNJOBST,

TABLE 2

*Two- and three-point crosses with group V markers*

| Zygote genotype and recombination percent | Parental combinations | Recombinations: Singles region 1 | Recombinations: Singles region 2 | Recombinations: Doubles regions 1 and 2 | Total and percent germination | Marker isolation numbers |
|---|---|---|---|---|---|---|
| + *b* *c* / *a* + + | +*bc* *a*++ | +++ *abc* | +*b*+ *a*+*c* | ++*c* *ab*+ | ... | (a) (b) (c) |
| + + *pl* / *lys-1* *me-3* + (37.3, 37.3) | 19 .. | 13 .. | 13 .. | 6 .. C=0.8 | 51 (*lys*⁺ only) 94% | 33933 36104 B118 |
| + + *am* / *iv-1* *sp* + (19.6, 4.3) | 70 .. | 18 .. | 4 .. | 0 .. | 92[V] (*iv*⁺ only) 88% | 16117 B132 47305 |
| + *sp* + / *iv-1* + *inos* (13.0, 17.4) | 9 23 | 1 5 | 5 3 | 0 0 | 52[V] 88% | 16117 B132 37401 |
| + *val* / *iv-1* + (0) | .. .. | 0 .. | .. .. | .. .. | 73*[P] 90% | 16117 45201 |
| + *sp* *inos* / *val* + + (19.0, 9.5) | 41 42 | 12 10 | 5 6 | 0 0 | 116[P] 97% | 45201 B132 37401 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| + *am* *inos*<br>*sp* + +<br>3.5 7.1 | 39 | 37 | 2 | 1 | 3 | 3 | 0 | 0 | 85[V]<br>85% | B132<br>47305<br>37401 |
| + *me-3* *asp*<br>*sp* + +<br>14.3 30.2 | 24 | 45 | 7 | 7 | 19 | 14 | 2<br>C=0.6 | 1 | 119[V]<br>75% | B132<br>36104<br>S1007 |
| + + *bis*<br>*hist-1* *inos* +<br>7.8 4.5 | 113 | 125 | 10 | 9 | 8 | 2 | 1<br>C=2.1<br>(>0.26) | 1 | 269<br>83% | C84<br>37401<br>B6 |
| + *me-3* *bis*<br>*hist-1* + +<br>7.6 1.4 | 58 | 74 | 4 | 7 | 1 | 1 | 0 | 0 | 145<br>48% | C84<br>36104<br>B6 |
| + + *pab-2*<br>*hist-1* *bis* +<br>7.8 9.7 | 47 | 39 | 3 | 4 | 5 | 4 | 0<br>C=1.3<br>(>0.03) | 1 | 103<br>31% | C84<br>B6<br>H193 |
| + + *pab-2*<br>*hist-1* *bis* +<br>1.7 9.5 | 58 | 45 | 1 | 1 | 8 | 3 | 0 | 0 | 116<br>72% | C84<br>B6<br>H193 |
| + + *bis*<br>*inos* *pab-1* +<br>1.1 4.0 | 90 | 77 | 1 | 1 | 5 | 2 | 0 | 0 | 176[P]<br>88% | 37401<br>1633<br>B6 |
| + + +<br>*inos* *pab-1* *bis*<br>1.8 3.7 | 241 | 239 | 3 | 5 | 11 | 7 | 0<br>C=3.0<br>(>0.08) | 1 | 507<br>94% | 37401<br>1633<br>B6 |
| + (+) *wl*<br>*inos* (*pab-1*) +<br>9.7 | 55 | 57 | 5 | 7 | .. | .. | .. | .. | 124[V]<br>83% | 37401<br>(1633)<br>B66 |
| + *pab-2* +<br>*me-3* + *asp*<br>13.9 0 | 29 | 39 | 8 | 3 | 0 | 0 | 0 | 0 | 79[P]<br>88% | 36104<br>H193<br>S1007 |
| + + *pl*<br>*me-3* *asp* +<br>24.4 0.6 | 55 | 62 | 16 | 22 | 1 | 0 | 0 | 0 | 156[V]<br>90% | 36104<br>S1007<br>B118 |
| + *pl*<br>*me-3* +<br>23.3 | 12 | 11 | 4 | 3 | .. | .. | .. | .. | 30[P]<br>100% | 36104<br>B118 |
| + *pl*<br>*asp* +<br>0 | 29 | 25 | 0 | 0 | .. | .. | .. | .. | 54[P]<br>90% | S1007<br>B118 |
| + +<br>*asp* *pl*<br>3.3 | 18 | 11 | 0 | 1 | .. | .. | .. | .. | 30[P]<br>100% | S1007<br>B118 |

Numbers of progeny are given in the body of the table. Progeny genotypes are not designated explicitly, but the genotype of each class can be determined from the order of presentation. The left-hand number of each pair of complementary classes represents the genotype that contains the plus allele of the leftmost marker. Alleles shown in parentheses in the genotype column were not scored or recorded, nor were classes of segregants where dashes replace numbers. Regions are numbered from left to right, and isolation numbers in the last column are listed in the same order. Crosses are tabulated in a sequence corresponding to the position of their leftmost markers in the linkage group. Where gene order is uncertain, one arbitrarily chosen order has been used consistently. C=coincidence. When C exceeds one, the minimum coincidence is also given from which the observed value does not deviate significantly (2.5 percent one-sided level).

* Germinating ascospores scored on minimal plate.

[P] Data of PERKINS.

[V] Data of VEATCH, from Cox 1956.

TABLE 3

*Four- and five-point crosses. Conventions as in Table 2. Coincidence values are based on pairs of crossovers both in double and in triple recombination classes*

| Zygote genotype, recombination percent, and marker isolation numbers | Parental combinations | | Recombinations: Singles Region 1 | | Singles Region 2 | | Singles Region 3 | | Singles Region 4 | | Doubles (Regions in parentheses) | | | | Triples | Total and percent germination |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| + *inos* *pab-1* *bis*<br>*lys-2* + + +<br>19.7 3.7 2.3<br>37101, 37401, 1633, B5 | 77 | 88 | 18 | 22 | 5 | 1 | 3 | 1 | .. | .. | 0 2 (1&2) C=1.3 (>0.15) | 0 1 (1&3) | .. .. | | 0 | 218<br>73% |
| + *inos* *pab-1* *asp*<br>*hist-1* + + +<br>10.0 3.3 8.9<br>C84, 37401, 1633, C123 | 36 | 34 | 3 | 6 | 3 | 0 | 4 | 4 | .. | .. | 0 | .. .. | .. .. | .. .. | 0 | 90<br>90% |
| + *inos* *pab-1* *bis* +<br>*hist-1* + + + *ad-7*<br>2.4 1.2 1.2 9.2<br>C84, 37401, 1633, B6, 44411 | 99 | 118 | 2 | 3 | 1 | 2 | 1 | 2 | 9 | 13 | 0 1 (1&4) | .. .. | .. .. | .. .. | 0 | 251<br>85% |
| +. *inos* *pab-1* *bis* +<br>*hist-1* + + + *asp*<br>7.1 9.5 11.8 11.8<br>C84, 37401, 1633, B5, C123 | 39 | 43 | 4 | 4 | 3 | 3 | 8 | 3 | 7 | 7 | 0 1 (1&2) C=1.2 (>0.03) | 2 1 (2&3) C=2.9 (>0.72) | 1 0 (2&4) | 0 1 (2,3,4) | | 127<br>64% |
| + *inos* *pab-2* +<br>*hist-1* + + *asp*<br>10.8 20.3 14.9<br>C84, 37401, H193, C123 | 13 | 29 | 2 | 4 | 6 | 8 | 7 | 3 | .. | .. | 1 0 (1&2) C=0.6 | 0 1 (1&3) | .. .. | | 0 | 74<br>25% |
| + + *me-3* *bis*<br>*inos* *pab-1* + +<br>0.4 2.2 0.8<br>37401, 1633, 36104, B6 | 114 | 110 | 0 | 1 | 2 | 3 | 1 | 1 | .. | .. | 0 | .. .. | .. .. | .. .. | 0 | 232<br>77% |
| + + *me-3* *bis*<br>*inos* *pab-1* + +<br>37401, 1633, 36104, B6 | .. | .. | .. | .. | .. | .. | .. | 19 | .. | .. | .. .. (1&2) | 0 .. (1&3) | 0 .. (2&3) | .. 0 (1,2,3) | | 19<br>(*me*$^+$ *bis*- only) |
| + + + *asp*<br>*inos* *pab-1* *bis* +<br>5.3 6.6 1.3<br>37401, 1633, B5, C123 | 40 | 26 | 3 | 1 | 1 | 4 | 1 | 0 | .. | .. | 0 | .. .. | .. .. | .. .. | 0 | 76<br>76% |
| + + + +<br>*inos* *pab-1* *bis* *asp*<br>2.1 4.2 24.8<br>37401, 1633, B6, S1007 | 42 | 27 | 0 | 1 | 0 | 2 | 12 | 11 | .. | .. | 0 1 (1&2) C=12.1 (>0.31) | 0 1 (2&3) C=1.0 | .. .. | | 0 | 97<br>97% |
| + + + *asp*<br>*inos* *pab-1* *bis* +<br>3.0 2.2 25.4<br>37401, 1633, B6, S1007 | 44 | 51 | 0 | 2 | 3 | 0 | 14 | 18 | .. | .. | 2 0 (1&3) | .. .. | .. .. | .. .. | 0 | 134<br>67% |

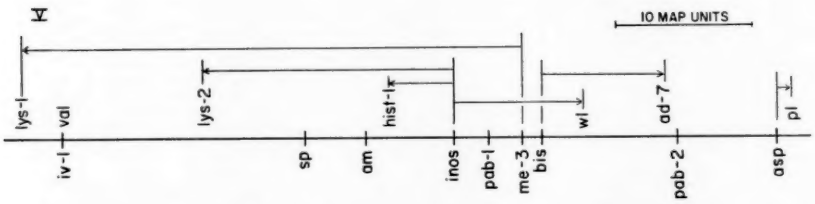


FIGURE 1.—Partial map of group V, summarizing the data from Tables 2 and 3. The sequence of loci written below the heavy line is based directly on 3-point data for all the genes involved. Above the heavy line, only the relationships indicated by arrows are based on 3-point data. Each arrow originates from the medial marker in the 3-point cross, and leads to a marker that was nonmedial. Interval lengths are imprecise owing to the variability of crossing over. The map is based solely on data from the present paper, except that the position shown for genes above the line may reflect the results of other workers in cases where our own data would require order to be decided arbitrarily or to be based only on quantitative 2-point data from different crosses. See text for sequences established by other workers, The centromere is located left of *iv-1*.

a number of sequences in group V have been reported or confirmed by other workers: centromere 10 *iv-1* 10 *hist-1* 7 *inos* 3 *pab-1* (GILES 1956); *iv-2 hist-1* 5.5 *inos* 0.9 *pab-1* 0.9 *me-3* 14.5 *asp*(1A7) (FREESE 1957a, b); centromere *sp am hist-1 inos pab-1* (BARRATT personal communication, 1956); *sp am inos* (PATEMAN 1958). Data in the present paper are consistent with these results.

The chief remaining uncertainties regarding gene order concern *lys-2* with respect to markers proximal to *inos*; *ad-7* and *wl* with respect to markers distal from *bis*; and *asp* and *pl* with respect to one another. The seriation shown for *pl* and *asp* depends on a single isolate.

All markers so far mapped in group V are in the same chromosome arm. (A possible exception is *lys-1* which in any case is close to the centromere.) Such an apparently non random distribution of genes might be due to the fact that distal markers such as *inos* have frequently been used for linkage tests.

Linkage of *lys-2* (37101) in V was first detected by GOOD (1952). *am* was first mapped by DR. R. W. BARRATT (personal communication).

*Status of new mutants:* 45201 is the first valine mutant to be mapped. (The first two digits are probably correct, but not completely certain.) No recombinants were observed in a cross between *val* and *iv-1* (16117), which requires both isoleucine and valine. *asp*(C123) and *asp*(S1007) show dissimilar crossover frequencies in several crosses. Direct evidence regarding allelism is not available. C123 is allelic with two other asparagine mutants, 1A7 and 67603 (DUBES 1953).

General aspects of the results reported here have been discussed by PERKINS (1959) in conjunction with comparable data from other linkage groups.

## SUMMARY

Multiple-point linkage data have been obtained on 15 loci, including five that are new, in group V of *Neurospora crassa*, using random segregants obtained by nonselective methods. All loci are in the same chromosome arm, with the possible exception of one which is close to the centromere. Chiasma interference is positive.

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# THE GENOTYPIC CONTROL OF KAPPA IN PARAMECIUM AURELIA, SYNGEN 4, STOCK 51[1]

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THE killer trait in all stocks of *Paramecium aurelia* which have been investigated has been found to depend upon the presence of a cytoplasmic factor, kappa, carried by killers but absent from sensitive animals (SONNEBORN 1943). Kappa is a DNA containing particle of about one micron in size (PREER 1950a; PREER and STARK 1953). When kappa disappears from the cytoplasm of a killer, the animal loses the ability to produce *P* particles (responsible for the killing action) and at the same time becomes sensitive to them (SONNEBORN 1946; PREER 1948).

The understanding of the relation of kappa to the nuclear genes of Paramecium has undergone changes with increasing knowledge and is further modified by the results to be presented in this paper. SONNEBORN (1943) early demonstrated the presence of a pair of alleles, *K* and *k*, which play a decisive role in the control of the maintenance of kappa particles. Under standard conditions of culture, kappa and the killer trait are maintained by animals of genotype *KK* and *Kk*, but they are lost by animals of genotype *kk*. When this was the only known locus related to the maintenance of kappa, the possibility of an intimate and direct relation between the allele *K* and kappa was entertained (SONNEBORN 1945). This possibility, first rejected on other grounds (SONNEBORN 1946), was soon rendered untenable by the discovery of a second locus (*S-s*) affecting the maintenance of kappa (SONNEBORN 1947b). Animals of genotype *KK SS* give rise to a "mixed" clone, that is a clone characterized by the presence of sensitive as well as killer lines of descent. Since the sensitives are killed by the action of *P* particles secreted by the killer animals, mixed clones are characterized by the occurrence within them of a specific type of autolethality. A clone of genotype *KK ss* behaves as a pure killer and produces no sensitives under standard conditions of culture. Mixed clones were obtained following crosses between the killer stock 51 and either of the sensitive stocks 29 or 32 in syngen 4 of *Paramecium aurelia*. Stock 51 carries the allele *s*, stock 29—and presumably stock 32—carry the allele *S*. As will appear, two such *S-s* loci have now been found and more are suspected of existing.

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The present paper is devoted mainly to one aspect of the problem of nuclear control of kappa: the number of loci involved and their interactions, both in their effect upon the maintenance of the particles in killers, and their establishment in previously sensitive animals. The nature of the effects of these genes on kappa is dealt with more directly elsewhere (BALBINDER 1957 and in preparation).

## MATERIALS AND METHODS

The following stocks of *Paramecium aurelia* from SONNEBORN's collection were employed: stock 31 of syngen 8; stocks 29, 51, d4–186, and d4–22 of syngen 4. All are sensitive except the killer stock 51. Stock d4–186 (referred to as stock 186 by DIPPELL 1950) carries gene *k* (from stock 29) but is otherwise virtually isogenic with stock 51; stock d4–22 carries the same *k* gene and also genes $H^{29}$ (for antigen H) and $D^{32}$ (for antigen D) from stocks 29 and 32, respectively, but is otherwise essentially isogenic with stock 51. Stocks 51 and 29 were the sources of the genes affecting kappa. Stock 31 was used as the detector of killing action. The stocks d4–186 and d4–22 were used mainly for control crosses, the latter when serotypic markers were desired. Serotypic markers were used to establish that true crosses were obtained and also to determine the cytoplasmic ancestry of the clones derived from each of the members of a conjugating pair. The antigens selected, 51H and 29H in some cases, and 51D and 32D in others can be readily recognized serologically. Heterozygotes for either the H or the D types can be easily distinguished from the respective homozygotes (SONNEBORN and BALBINDER 1953, for the H types; SONNEBORN *et al.*, unpublished, for the D types). When these markers were not available, nonallelic antigen markers, such as 51A and 51B (SONNEBORN 1951) were employed. True crosses were distinguished from selfing in some cases by the segregation of known markers (such as *K-k*) in the autogamous $F_2$.

The culture medium used for most of the work reported in this paper was a baked lettuce infusion inoculated with *Aerobacter aerogenes*, incubated at 27°C for 24 hrs and finally adjusted to a pH of approximately 6.5 before use. In some cases, a dehydrated lettuce infusion (Difco) and in a few others a commercial rye infusion (Cerophyll) were used as culture media. In all cases *A. aerogenes* was used as food organism. The differences in culture media used had no detectable effect on the results reported in this paper.

The methods employed for making crosses, avoiding and inducing autogamy, testing for killing ability, mating type and serotype, and detecting cytoplasmic exchange were those described by SONNEBORN (1950).

### *Experimental method*

Following a cross, each of the exconjugants was isolated separately into fresh culture fluid and allowed to divide once. One of the daughter cells was tested for serotype, to make sure each pair of exconjugants had yielded one line of each of the serotypically diverse parents. The remaining animal was allowed to develop

into a clone. By a series of daily reisolations, clones derived from exconjugants ($F_1$ clones) were allowed to undergo about 20 rapid fissions. After the 20th fission, an $F_2$ generation by autogamy was obtained, each ex-autogamous individual being isolated into a separate culture slide and allowed to produce a clone. After autogamy, only homozygotes are obtained (SONNEBORN 1939, 1947a). Both the $F_1$ and $F_2$ clones were tested for killing ability and serotype, and observed for the presence of autolethality after completing 10–11 fissions. Clones were classified as sensitives if killing was observed only in mixtures of the unknown with killers; as killers if killing was observed only in mixtures of the unknown with sensitives; as mixed if killing was observed in both types of mixtures as well as in samples of the unknown by itself.

$F_2$ clones testing as sensitives at the 10th fission test were discarded, killer and mixed clones being retained for further analysis. The latter were observed under different conditions of culture, as described below, in order to differentiate various types of mixed clones from each other and from pure killers.

*Criteria used for identifying the different types of $F_2$ clones:* The characteristics of different types of mixed clones and pure killer clones under a variety of conditions of culture have been described in detail elsewhere (BALBINDER 1957). They will only be briefly summarized here.

Three types of methods were used:

1. Serial subcultures in depression slides at 27°C using ten animals to start each subculture. Generally these were carried through six successive subcultures (about 50 fissions after autogamy). Tests for killing ability and estimates of the percentage of sensitives present were carried out on each subculture.

2. Mass cultures in tubes at 27°C for long periods of time, with frequent testing for killing and autolethality.

3. Exposure to conditions favoring loss of kappa, such as high (35°C) or low (14°C) temperature. At 35°C mass cultures of each clone were allowed to starve for four days, with samples of about 100 animals being withdrawn periodically and subcultured slowly at 27°C. Kappa in killers is progressively destroyed or inactivated in the cytoplasm at temperatures above 33.8°C (SONNEBORN 1947b). However, as long as a single kappa particle remains and is able to reproduce it will restore the normal number of particles after a short period of slow growth at 27°C (PREER 1948). At 14°C the animals were grown with excess food, so that they would reproduce at their maximal rate (one fission per day at this temperature). Under these conditions, kappa multiplies at a slower rate than the paramecia and is gradually diluted out (CHAO 1954).

Three main types of clones could be distinguished by these three methods. The first two methods allow for distinction of the three types of clones on the basis of the frequency of occurrence of autolethality and the percentage of sensitives present at each occurrence, as well as the eventual fate of the killer trait: i.e., whether it is totally lost or retained. The third method differentiates among the three types of clones by the rates at which they become completely sensitive. Loss of the killer trait in mixed clones is due to the disappearance of kappa from

the cytoplasm, possibly by actual destruction of the particles (BALBINDER 1957). The three types of clones and their behavior upon culture by each of the three methods were the following:

1. MS (mixed-sensitive) clones. These showed frequent and abundant autolethality by methods 1 and 2; lost kappa completely, the whole clone becoming sensitive after long culture in tubes by method 2, and lost kappa rapidly at both 14°C and 35°C (method 3).

2. M clones (permanently mixed). These sporadically produced sensitives when cultured by methods 1 and 2, never lost their killing ability completely after long periods of culture in tubes by method 2, and lost kappa at intermediate rates by method 3. By cultivation with different combinations of fission rate and temperature, two subgroups of M clones, $M_1$ and $M_2$, could be differentiated (BALBINDER 1957 and in preparation). On testing, these were shown to be genotypically different (see Table 1).

3. Pure killers. These always tested as killers by methods 1 and 2 and lost kappa slowly by method 3. The behavior of pure killer segregants in crosses involving mixed clones was paralleled by pure killer controls.

For each cross, the postautogamous $F_2$ clones were cultured in parallel series employing two or all three of these methods or minor variations thereof. Thus, the identification of each clone is based on at least two independent criteria. In all experiments, control crosses between the killer or killers used for the experimental crosses and either one of the sensitive stocks d4–186 or d4–22 were performed simultaneously.

## RESULTS

*Genetic analysis of mixed clones—Number of loci relevant to kappa distinguishing stock 51 from stock 29; their effect on kappa maintenance in homozygotes; their synergism:* The homozygous $F_2$ segregants derived from an $F_1$ killer after crossing pure killers of stock 51 by pure sensitives of stock 29 gave about 50 percent pure sensitives (*kk*) as expected. The remaining 50 percent were *KK* pure killers and mixed clones never deviating significantly from one fourth MS to three fourths non-MS (i.e., $M_1$ plus $M_2$ plus pure killers) clones. In six backcrosses to stock 51 killers, selecting an MS segregant from each successive autogamous generation for backcrossing to stock 51, no pure sensitives were obtained after autogamy but the ratio of MS to non-MS clones remained 1:3. In cases where the $F_2$ clones were more closely observed, four phenotypic groups (MS, $M_1$, $M_2$, pure killer) were discernible (BALBINDER 1957) in a ratio of 1:1:1:1. These observations suggested that genes at two loci, segregating independently from each other and from the *K-k* alleles were responsible for the production of mixed clones. Following SONNEBORN's (1947b) system of nomenclature the alleles at each one of these two loci were designated $S_1$ and $S_2$ for loss of kappa and, respectively, $s_1$ and $s_2$ for kappa maintenance. The phenotypes and genotypes of each class could then be written as follows:

| Phenotype | Genotype |
|---|---|
| Pure killer | $KK\ s_1s_1\ s_2s_2$ |
| M $\{M_1$ | $KK\ S_1S_1\ s_2s_2$ |
| M $\{M_2$ | $KK\ s_1s_1\ S_2S_2$ |
| MS | $KK\ S_1S_1\ S_2S_2$ |

This hypothesis was confirmed by a number of breeding experiments, the results of which are given in Table 1. On performing the genetic analysis of

TABLE 1

*Crosses between MS clones, M clones, and pure killers. Ratios observed in the postautogamous $F_2$ generation following each cross. In some cases all segregating genotypic classes could be scored, in others only some. All clones involved in these crosses were homozygous* KK *but varied with respect to the alleles at the loci* $S_1$-$s_1$ *and* $S_2$-$s_2$. *For further explanation see text*

| Cross | | | Autogamous $F_2$ | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Observed | | | | Expected | | | | | | |
| | | | | M | | | | M | | | | | |
| Phenotype | Genotype | No. of exp. | Killer | $M_1$ | $M_2$ | MS | Killer | $M_1$ | $M_2$ | MS | Total | $\chi^2$ | P |
| | | | $s_1s_1$ $s_2s_2$ | $S_1S_1$ $s_2s_2$ | $s_1s_1$ $S_2S_2$ | $S_1S_1$ $S_2S_2$ | $s_1s_1$ $s_2s_2$ | $S_1S_1$ $s_2s_2$ | $s_1s_1$ $S_2S_2$ | $S_1S_1$ $S_2S_2$ | | | |
| Killer × MS | $s_1s_1\ s_2s_2$ × $S_1S_1\ S_2S_2$ | 2 | 19 | 14 | 23 | 19 | 18.75 | 18.75 | 18.75 | 18.75 | 75 | 2.17 | 0.6 |
| | | 1 | 42 | 63 | | 37 | 35.5 | 71.0 | | 35.5 | 142 | 2.14 | 0.4 |
| | | 6 | 385 | | | 116 | 375.75 | | | 125.25 | 501 | 0.91 | 0.35 |
| Killer × M ($M_1$ or $M_2$) | $s_1s_1\ s_2s_2$ × $S_1S_1\ s_2s_2$ or $s_1s_1\ S_2S_2$ | 2 | 45 | 41 | | 0 | 43 | 43 | | 0 | 86 | 0.18 | 0.65 |
| $M_1 \times M_2$ | $S_1S_1\ s_2s_2$ × $s_1s_1\ S_2S_2$ | 1 | 113 | | | 32 | 108.75 | | | 36.25 | 145 | 0.66 | 0.4 |
| $M_1 \times M_1$ or $M_2 \times M_2$ | $S_1S_1\ s_2s_2$ × $S_1S_1\ s_2s_2$ or $s_1s_1\ S_2S_2$ × $s_1s_1\ S_2S_2$ | 1 | 148 | | | 0 | 148 | | | 0 | 148 | ... | ... |
| $M_1 \times MS$ or $M_2 \times MS$ | $S_1S_1\ s_2s_2$ or $s_1s_1\ S_2S_2$ × $S_1S_1\ S_2S_2$ | 1 | 51 | | | 45 | 48 | | | 48 | 96 | 0.38 | 0.55 |

mixed clones, the experiments could be designed so as to distinguish all segregating genotypic classes or only some of them. In general MS segregants were readily recognizable from M and killer segregants, and most experiments were therefore designed to yield a ratio of non-MS (killer and M) clones:MS clones. A few experiments were designed to distinguish all phenotypes. As can be seen, in all cases the observed results were in agreement with expectations. They agree with SONNEBORN's (1947b) observation that stock 29 carries a gene which brings about a permanently mixed condition when introduced into stock 51 killers. It is likely that SONNEBORN's *S* gene is identical with either $S_1$ or $S_2$.

Tests for additional genes carried by stock 29 which might be involved in the production of mixed clones failed to reveal any (BALBINDER 1957). It is possible, however, that genes at other loci may exist if their individual effects are extremely small and a large number of them is necessary for a detectable manifestation.

No effects of mating type or cytoplasmic exchange between mates on the $F_2$ ratios were observed, although these were looked for.

Individually, judging by their ability to eliminate kappa on culture at extreme temperatures (method 3), $S_1$ acts more effectively than $S_2$ (BALBINDER 1957). It is clear, from the results presented, that the individual effects of each, $S_1$ and $S_2$, are cumulative in the double homozygotes (MS clones). The question now arises as to whether synergism also exists between these alleles and the gene *k*. What would happen in combinations between $S_1$ and/or $S_2$ on one hand, and *k* on the other? Since *kk* animals develop into pure sensitive clones, this problem could only be studied in *Kk* heterozygotes. This is discussed in the next section.

*Effects of alleles at the three loci connected with kappa maintenance when one or more loci are heterozygous:* The relevant data are summarized in Table 2. The production of sensitive animals in $F_1$ clones derived from the killer exconjugants at about ten fissions past their origin at conjugation, following crosses between killers and sensitives of different genotypes, was used as a criterion to detect possible effects of various gene changes from the pure killer genotype *KK* $s_1s_1$ $s_2s_2$. The substitution of *k* for one *K* does not in itself accomplish this (cross 11), nor does the replacement of up to three of the four *s* genes for their *S* alleles (crosses 7 and 8). However, the substitution of both *k* for one *K* and an *S* for one *s* did yield sensitives within ten fissions in nearly all clones (15/17, see crosses 4 and 5). Hence it appears that one *k* in conjunction with one *S* can accomplish what neither alone can. It would thus seem that *k* and the *S* alleles act synergistically.

CHAO (1953) showed that for kappa maintenance the parental genotype of the animals is effective for the first eight fissions following nuclear reorganization, the $F_1$ genotype becoming operative after that. Although the data in Table 2 indicate that the prezygotic genotype of the killer parent has an effect on determining the proportion of clones showing sensitive animals, they make it clear that the differences observed among the various $F_1$'s cannot be accounted

TABLE 2

*Killer and mixed clones observed at the tenth postzygotic fission among the descendants of the killer exconjugants in crosses between killers and sensitives of different genotypes*

| Cross no. | Prezygotic genotype of killer | $F_1$ genotype | No. of experiments | Total no. $F_1$ tested | No. killers | No. mixed | Percent mixed |
|---|---|---|---|---|---|---|---|
| 1 | *KK* $s_1s_1$ $s_2s_2$ | *Kk* $S_1s_1$ $S_2s_2$ | 7 | 148 | 99 | 49 | 32.0 |
| 2 | *KK* $S_1S_1$ $s_2s_2$ | *Kk* $S_1S_1$ $S_2s_2$ | 1 | 11 | 0 | 11 | 100.0 |
| 3 | *KK* $s_1s_1$ $S_2S_2$ | *Kk* $S_1s_1$ $S_2S_2$ | 1 | 6 | 1 | 5 | 83.0 |
| 4 | *KK* $S_1S_1$ $s_2s_2$ | *Kk* $S_1s_1$ $s_2s_2$ | 1 | 7 | 0 | 7 | 100.0 |
| 5 | *KK* $s_1s_1$ $S_2S_2$ | *Kk* $s_1s_1$ $S_2s_2$ | 1 | 10 | 2 | 8 | 80.0 |
| Totals | | | 11 | 182 | 102 | 80 | ... |
| 6 | *KK* $s_1s_1$ $s_2s_2$ | *KK* $S_1s_1$ $S_2s_2$ | 7 | 116 | 115 | 1 | 0.8 |
| 7 | *KK* $S_1S_1$ $s_2s_2$ | *KK* $S_1S_1$ $S_2s_2$ | 1 | 10 | 10 | 0 | 0.0 |
| 8 | *KK* $s_1s_1$ $S_2S_2$ | *KK* $S_1s_1$ $S_2S_2$ | 1 | 9 | 9 | 0 | 0.0 |
| 9 | *KK* $S_1S_1$ $s_2s_2$ | *KK* $S_1s_1$ $s_2s_2$ | 1 | 11 | 11 | 0 | 0.0 |
| 10 | *KK* $s_1s_1$ $S_2S_2$ | *KK* $s_1s_1$ $S_2s_2$ | 1 | 18 | 18 | 0 | 0.0 |
| Totals | | | 11 | 164 | 163 | 1 | ... |
| 11 | *KK* $s_1s_1$ $s_2s_2$ | *Kk* $s_1s_1$ $s_2s_2$ | 11 | 160 | 160 | 0 | 0.0 |

for by the effect of the parental genotype. A comparison of crosses 1 through 5 shows that in all cases where the killer parent was homozygous for either $S_1$ or $S_2$ (crosses 2 through 5) most of the $F_1$ clones (31/34 or 91 percent) yielded sensitives within ten fissions. On the other hand, when the killer parent was homozygous for both $s_1$ and $s_2$, a much smaller fraction of the clones 49/148 or 32 percent did so (cross 1). This was true despite the fact that the $F_1$ of the latter cross contained one dose of both $S_1$ and $S_2$ while in two of the former crosses (4 and 5) the $F_1$ contained one dose of either $S_1$ or $S_2$, not both. However, a comparison between crosses 1, 6, and 11, where the prezygotic genotype was the same, shows that the percentage of mixed clones observed was higher for cross 1 than for the other two, and this is correlated with the $F_1$ genotype. Since the effects of the parental genotype were only manifested in the cases where the $F_1$ genotype was propitious for the production of sensitive animals (i.e., crosses 1 through 5 as compared to crosses 6 through 10), it is possible that some sort of synergism also exists between prezygotic and postzygotic genotypes, as well as between alleles at the three different loci within the same genotype. Unfortunately, the number of $F_1$ clones analyzed is too small to conclude that such an interaction does exist. This deserves further investigation.

The possibility of effects of diverse prezygotic killer genotype make it difficult to infer whether, in the cases analyzed, the magnitude of the effect of *S* genes on kappa is proportional to their total number in combination with one dose of *k*. To settle this point, experiments are required in which the same killers are crossed to sensitives of different genotypes with respect to the *K-k* and *S-s* loci.

Dosage dependent effects have been reported for the *K-k* alleles. CHAO (1953) showed that *Kk* heterozygotes have one half as much kappa as *KK* homozygotes of the same mating type. Dosage dependent effects have also been observed for the *S* alleles. Thus, MS clones (*KK* $S_1S_1$ $S_2S_2$) showed the presence of some sensitives within 10–17 fissions and large numbers of sensitives in 100 percent of the cases by the 30th fission (BALBINDER 1957), while *KK* $S_1s_1$ $S_2s_2$ heterozygotes followed for more than 30 fissions never showed the presence of sensitive animals, with the single exception shown in Table 2 (cross 6). A single such exception is of dubious significance. These facts, together with the results presented in the preceding section and elsewhere (BALBINDER 1957) suggest that the genes controlling the maintenance of kappa constitute a set of multiple factors which lack dominance and act synergistically but unequally. The order of effect on loss of kappa is: $k>S_1>S_2$, and $k>S_1$ plus $S_2$. In homozygous condition, *kk* can be thought of as epistatic to all the alleles at the $S_1$-$s_1$ and $S_2$-$s_2$ loci, since *kk* $s_1s_1$ $s_2s_2$ animals are sensitive. According to CHAO (1953), kappa is completely eliminated in the latter between the eighth and 15th fissions following the acquisition of the *kk* genotype. Whether in homozygotes for *kk* containing at least one of the *S* alleles, kappa is eliminated before the eighth fission remains unknown.

It should be mentioned that the stage at which the observations were made, the tenth postzygotic fission, did have an influence on the total number of mixed clones observed. This is illustrated by the behavior of MS clones, mentioned above. However, for comparing the effects of the different genotypes on kappa maintenance, the tenth fission test proved adequate.

The differences observed between the different groups of crosses were not influenced by the amounts of cytoplasm exchanged at conjugation between the mates (BALBINDER 1957).

*The establishment of kappa in previous sensitives: role of genes* k, $S_1$, *and* $S_2$: CHAO (unpublished, reviewed by SONNEBORN 1959) showed that the *k* gene affects kappa establishment in homozygotes. He observed cytologically that in conjugating pairs of *Kk* killers × *kk* sensitives, kappa was transferred to the *kk* mate when sufficiently broad cytoplasmic bridges were formed, but quickly disappeared when the genotype remained *kk*. The fact that $S_1$ and $S_2$, as well as *k*, affect kappa maintenance and act synergistically raises the question of whether the *S* genes may also affect kappa establishment. A suggestion that this might be the case is found in the behavior of stock 169, a syngen 4 killer isolated from nature which in some ways parallels the behavior of mixed clones. Stock 169 shows spontaneous autolethality in laboratory cultures (REISNER, unpublished) TALLAN (1956, 1957) conducting studies on the establishment of kappa in sensitive animals after exposure to killer breis, showed that kappa becomes established less readily in stock 169 sensitives than in sensitives of stock 51. He showed these differences to be independent of the type of kappa used and dependent on nuclear factors not allelic with *K-k*.

SONNEBORN (1943, 1946) has shown that kappa can be transmitted from a

killer to a sensitive animal during conjugation, if a cytoplasmic bridge forms between the mates and persists for a certain length of time. For example, in crosses between certain killers and sensitives, SONNEBORN (1946) found no transfer of kappa if the bridge lasted less than three minutes; transfer of kappa in only some pairs if the bridge lasted from 3½ to 30 minutes, and invariable transfer of kappa followed by its establishment in the sensitive mate (thus transforming it into a killer) if the cytoplasmic bridge lasted over 30 minutes. Thus, the amount of cytoplasmic exchange must be taken into account when considering the role of the genes in the control of kappa's ability to become established in sensitives.

The results of several experiments are summarized in Table 3. The number of clones derived from the sensitive mate, following several killer × sensitive crosses, in which kappa became established after ten postzygotic fissions as well as those where it did not, are given for different periods of persistence of the cytoplasmic bridge. In cases where a relatively small amount of cytoplasm was exchanged between the mates (bridges lasting 0–5 minutes, for instance), allowing for few kappa particles to enter the cytoplasm of the sensitive mate, it is possible that these were only transmitted to one of the first fission products, and that this one was sacrified to perform a serotype test. This could have led to an underestimate of the total number of $F_1$ clones for each cross where kappa was capable of becoming established. However, since all $F_1$ clones were treated identically, comparison between them to determine the relative ability of different genotypes to influence the establishment of kappa is possible. It is also likely that in some cases the kappa level remained low for the first few fissions after conjugation, with occasional individuals within the clone receiving no kappa

TABLE 3

*Relationship between genotype, cytoplasmic exchange (as measured by delay in separation at the paroral cone region following conjugation) and establishment of kappa. Number of clones, derived from the sensitive exconjugant of killer × sensitive crosses, showing no killing ability (sensitives) and killing ability (killers and mixed) after ten postzygotic fissions*

| Time of sep. at paroral cone (minutes) | Prezygotic genotype of sens. parent | *kk* $S_1S_1$ $S_2S_2$ | | | | *kk* $s_1s_1$ $s_2s_2$ | | | | *KK* $S_1S_1$ $S_2S_2$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Genotype of $F_1$ | *Kk* $S_1s_1$ $S_2s_2$ | | | | *Kk* $s_1s_1$ $s_2s_2$ | | | | *KK* $S_1s_1$ $S_2s_2$ | | | |
| | | S | K&M | Total | Percent K&M | S | K&M | Total | Percent K&M | S | K&M | Total | Percent K&M |
| 0–5 | .. | 36 | 1 | 37 | 2.7 | 70 | 17 | 87 | 19.5 | 6 | 8 | 14 | 57.0 |
| 6–23 | .. | 44 | 6 | 50 | 12.0 | 11 | 18 | 29 | 61.6 | 10 | 18 | 28 | 64.3 |
| >23 | .. | 6 | 2 | 8 | 25.0 | 1 | 3 | 4 | 75.0 | 0 | 31 | 31 | 100.0 |
| Totals | | 86 | 9 | 95 | ... | 82 | 38 | 120 | ... | 16 | 57 | 73 | ... |

The genotype of the killer parent was always *KK* $s_1s_1$ $s_2s_2$, the sensitive parent's genotype being different in each case as shown in the table. Symbols: K=killers; M=mixed; S=sensitives.

at the time of cell division. These would develop into sensitive sublines within a predominantly killer clone, as fission rate decreased towards the tenth fission due to limiting food supply. Under such conditions kappa could reproduce faster than the animals and reach killer concentrations in those individuals possessing particles. Clones containing killer and sensitive animals would test as mixed, and a number of these were observed. Since in these clones kappa reproduction did take place to the extent that killer animals were present, kappa was considered to have become established in them.

As can be seen in Table 3, in all cases the genotype of the killer mate was the same, but the genotypes of the sensitives varied at the *K-k* and *S-s* loci. It is therefore likely that in some of the crosses (particularly those where the sensitive mate was homozygous *kk*), the reduced ability of kappa to become established might have been due to the influence of the parental genotype during the early stages of establishment (before the eighth fission). However, from the fact that kappa did become established in clones derived from *kk* sensitives after the *K* gene was introduced, even when the cytoplasmic bridges lasted a short time, it is clear that the prezygotic genotype is not in exclusive control of kappa establishment.

Regardless of whether the effects of the prezygotic or zygotic genotype predominate, two facts emerge clearly from a consideration of Table 3: (1) the *S* genes do have an effect on kappa establishment and, (2) the action of the *k* and *S* genes in affecting kappa establishment is synergistic. This can be seen by comparing the percentage of establishment in clones of all three genotypes for the same duration of the cytoplasmic bridge. Thus, for example, for bridges lasting 0-5 minutes, kappa was established in 57 percent of the *KK* $S_1s_1$ $S_2s_2$ clones, 19.5 percent of *Kk* $s_1s_1$ $s_2s_2$ clones and 2.7 percent of *Kk* $S_1s_1$ $S_2s_2$ clones. In general, independently of the duration of the bridge, establishment was significantly lower in clones heterozygous at all three loci than in those heterozygous at either the *K-k* or *S-s* loci. Clearly then, the $S_1$ and $S_2$ alleles inhibit kappa establishment, at least when they are in combination with *k*. Whether $S_1$ and $S_2$ by themselves have a detectable effect on kappa establishment cannot be determined since the data necessary for a proper comparison (establishment in animals of genotype *KK* $s_1s_1$ $s_2s_2$) are lacking thus far. It is apparent that *k* by itself allowed for less kappa establishment than $S_1$ and $S_2$ together. This is certainly true for those cases where very little cytoplasm was exchanged (0-5 minutes delay); the differences observed for longer delays may not be significant.

As Table 3 shows, the *k* and *S* genes do not control establishment of kappa through an effect on the duration of the cytoplasmic bridge but must act after kappa has entered the cytoplasm. This is in full agreement with CHAO's observations mentioned before. The results also point to a relation between the amount of cytoplasm exchanged and genotype with respect to establishment. Thus, for $F_1$ genotype *Kk* $S_1s_1$ $S_2s_2$ kappa was established in 12.0 percent of the clones derived from sensitives when the bridges persisted for 6-23 minutes, while for $F_1$ genotype *Kk* $s_1s_1$ $s_2s_2$ establishment took place in 19.5 percent of the clones

derived from sensitives when the bridges persisted for only 0-5 minutes. Whether establishment in these cases depended on the total number of kappa particles entering the cytoplasm or on different amounts of an essential substance such as, for instance, the cofactor described by TALLAN (1956, 1957), is not known.

We can conclude from these results that the *k* and *S* genes act synergistically in controlling kappa's ability to become established in the cytoplasm of previously sensitive animals, in a manner similar to their control of kappa's stability in the cytoplasm of killers. Their effects again appear to be unequal, with $k > S_1$ plus $S_2$. Whether the magnitude of the effect is proportional to the total number of *S* genes in combination with *k* remains to be investigated.

## DISCUSSION

The results presented in this paper confirm and extend SONNEBORN's (1947b) observations showing that the role of the nucleus in controlling the killer trait is not confined to the *K*-*k* locus. Two more loci, $S_1$-$s_1$ and $S_2$-$s_2$ have been demonstrated, one of them probably identical with SONNEBORN's *S*-*s* locus. Alleles at all three loci act synergistically both in kappa elimination and establishment. Other cases of hereditary traits controlled by multiple factors have been reported for *Paramecium aurelia* (KIMBALL 1949; PREER 1950b).

Further investigation may reveal that more than the three loci demonstrated thus far are connected with the control of kappa. Some indications have already been found. In crosses between stock 51 killers and stock 32 sensitives for example, the instability of kappa gradually increased in backcrosses with approaching isogenicity to stock 32 (SONNEBORN, unpublished). Another sensitive stock in syngen 4, stock 203, produces no sensitive $F_2$ clones at the tenth postautogamous fission following a cross to a killer, but 100 percent of the segregants show autolethality (SIEGEL, unpublished).

The presence, in certain stocks, of genes with small individual effects such as $S_1$ and $S_2$, is an indication that, at least in some cases, sensitives could arise in nature from killers. Large mutations, such as $K \rightarrow k$ for example, might be eliminated after the first autogamy as they would render the homozygotes sensitive to *P* particles, while mutations with a smaller effect would be able to spread through the population. In such an event, changes in the environment (such as a decrease in temperature) by enhancing the expression of the mutated gene or genes, could bring about the elimination of kappa from all individuals in the population (as in the case of MS clones). Once the whole group becomes sensitive, large mutations would have a chance of becoming established. The cases of stock 169, mentioned earlier, and stock 203, are interesting in this connection since both are homozygous for *K* while apparently possessing other genes, with small individual effects, which are antagonistic to kappa. SONNEBORN and SCHNELLER (unpublished) have found that of 27 syngen 4 stocks isolated from nature, 17 are homozygous for *K* and 10 for *k*. Of the former, one third show autolethality in crosses to killers and may have genes similar to $S_1$ and $S_2$. The only *kk* stocks which have been investigated, stocks 29 and 32 carry *S*-like genes.

In syngen 2, no case has been found where the killer trait is under the control of a single pair of alleles similar to *K-k*. No indications exist, either, for the presence of *S*-like genes. SONNEBORN (1959) points out that all tested syngen 2 stocks have a genetic constitution favorable to kappa. The syngen 2 situation needs more extensive investigation.

*The relation between kappa and the genes controlling its maintenance:* The results presented in this paper showing that alleles at the *K-k* and *S-s* loci act synergistically in controlling both kappa maintenance and establishment, as well as the fact mentioned earlier that the alleles at each locus appear to exhibit no dominance, can be interpreted as meaning that kappa is controlled by a set of multiple factors whose individual effects are unequal but whose combined effects are cumulative, each gene contributing a certain amount, the effects of alleles at the three loci being similar in nature. This interpretation is strengthened by the fact that both in the case of the *S* genes (BALBINDER 1957) and in the case of *k* (CHAO 1953), kappa is eliminated abruptly, possibly by direct destruction in the cytoplasm. BEALE (1954), on the basis that kappa loss in *kk* homozygotes is abrupt, has suggested that the control exercised by gene *K* is not through the supply of some essential substance, but through the maintenance of a cytoplasmic state favorable for kappa reproduction. Kappa is known to be destroyed by a heterogeneous array of agents (antibiotics, heat, radiation, etc.; see SONNEBORN 1959, for review), and it is not impossible that some of the alleles involved in the control of kappa are responsible for the production, or accumulation in the cytoplasm, of substances which are harmful to kappa. On the other hand, as SONNEBORN (1959) has pointed out, the striking relation between dosage of the gene *K* and the kappa concentration, suggests that this allele might supply an essential requirement for kappa reproduction, the allele *k* being essentially inactive, and the alleles at both *S* loci acting in a completely different fashion. At the present time, no decisive evidence is available in favor of either view. It is clear nevertheless, as PREER (1957) has pointed out, that the manner of elimination of kappa by alleles at the three loci indicates that a more complex mechanism than merely supplying or withholding of a substance required for kappa reproduction is involved in kappa control.

The action of the *S* loci provides some insight into the results reported by TALLAN (1956, 1957) on stock 169. He found that this stock yields fewer killers than does stock 51 when sensitives of both stocks are exposed to infection by the same preparations of kappa, this difference being due to genes at one or more loci not linked to *K-k*. These results, together with the fact mentioned earlier that killers of stock 169 show autolethality, i.e., they produce sensitive animals, can be interpreted in the light of the knowledge of the *S* loci. Stock 169 is known to carry *K*; it may also be assumed to carry one or more *S*-like loci which are responsible both for the autolethality observed and the lesser success with attempted infection. This, of course, needs direct testing. TALLAN (1957 and in press) believes that the differences between stocks 51 and 169 in their susceptibility to infection are due to a decreased ability of kappa to become established in stock 169 after

penetration. Thus, the stock 169 genes influencing infection seem to act in a fashion similar to genes *k*, $S_1$ and $S_2$ in preventing kappa from becoming established after it has entered the cytoplasm.

TALLAN (1957) demonstrated a cofactor necessary for the establishment of kappa, but not for its uptake, in the breis of killers. On centrifugation of the brei, this cofactor could be separated from the infective unit (kappa). Whether this cofactor, the nature of which is unknown, is only necessary for the initial steps of establishment or whether it is a constant requirement for kappa maintenance is not known. The fact that it is present in killer breis may be an indication that it is always necessary. Since TALLAN reports that fresh culture fluid also enhances infection, although to a much smaller extent than the supernatant fraction of the brei (which contains the cofactor), it is possible that the cofactor is originally supplied by the medium and stored in the cytoplasm of killer animals, where it would be present in high concentration. Nevertheless, TALLAN's finding raises the question of whether any connection exists between the cofactor and the genes controlling the maintenance of kappa.

*The role of the cytoplasm and the question of "sigma":* SONNEBORN (1947b), in his initial work on the gene *S*, was led to postulate a cytoplasmic factor "sigma." Sigma was invented to account for two observations:

1. the coexistence of persistent sensitive and persistent killer sublines of descent in clones of a single genotype (*KK SS*); and

2. the transmission by these sensitive sublines, through their cytoplasm, of something which, when introduced abundantly into killers during conjugation, resulted in the production of sensitive descendants.

This something was called "sigma"; it was assumed to compete with kappa. This competition sometimes resulted in the disappearance of kappa (in pure sensitive sublines). The question of whether sigma was produced by gene *S*, or was merely maintained by it as kappa is by *K* was left open but the latter was indicated by the existence of pure killer sublines. All of SONNEBORN's comments were set forth as preliminary and tentative, and the full data have never been published.

In the present work, no attempt was made to repeat the studies of SONNEBORN on which the postulation of "sigma" was based. That is, sensitives from M clones (presumably *KK* $s_1s_1$ $S_2S_2$) were not crossed to killers to discover whether cytoplasmic transfer had an effect on the killer mate; i.e., the production of sensitives after ten postzygotic fissions. However, sensitives from MS clones (*KK* $S_1S_1$ $S_2S_2$) were studied in this way. No effect of the cytoplasm was found, the results being entirely explainable by an effect of the genotype. Similarly, the effects of cytoplasmic exchange between MS and killers on the $F_2$ ratios of such crosses were studied and pairs where the exconjugants had undergone no detectable exchange were compared to pairs where the mates had fused to form double animals. The results of these experiments were clear and unequivocal: no cytoplasmic effect was observed, the results being strictly accountable by the presence or absence of the *S* genes. This means that, whatever it is in mixed clones which is responsible

for the elimination of kappa, it must be gene initiated. Thus, while the observations do not justify the conclusion that "sigma" does not exist, they do not require its existence and can be fully explained by the action of the genes without resort to an additional cytoplasmic factor.

The cytoplasm, however, must play some role since this is where the kappa particles are located and it is difficult to see how the genes may affect them without in some way affecting the cytoplasm first. Some of the observations reported here and elsewhere (BALBINDER 1957), such as the fact that MS clones always lose kappa after long periods at 27°C, and the apparent synergism between the prezygotic and zygotic genotype on kappa elimination mentioned in connection with Table 2, may very well mean that there is an accumulation in the cytoplasm of products of the action of the *S* genes which create conditions unfavorable to kappa. These conditions may persist and become intensified when the proper genes are present, but may disappear very quickly if these are replaced by their alleles. It is possible that some of these products may be transmitted through cytoplasmic bridges at conjugation, but would be lost in the presence of the wrong genotype. The role of the cytoplasm in the production of mixed clones is still in need of thorough investigation, and may yield some important clues as to the nature of the relation between kappa and the genotype.

## SUMMARY

1. In syngen 4 of *P. aurelia* the maintenance of kappa in the cytoplasm of killers is controlled by genes at three loci: $K$-$k$; $S_1$-$s_1$; and $S_2$-$s_2$. At each locus two alleles exist: one favoring and the other opposing the maintenance of kappa.

2. The same loci control the maintenance of kappa in killers and its ability to become established in sensitives after it is introduced through cytoplasmic bridges.

3. Both in their effects on maintenance as well as establishment, the actions of the alleles at the three loci are cumulative (synergistic) although their individual effects differ, i.e., $K$ is more effective than $s_1$ and $s_2$ in favoring kappa, and $k$ is more effective than $S_1$ and $S_2$ in opposing it.

The relation between kappa and the genes controlling its maintenance is discussed.

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# NON-HOMOLOGOUS PAIRING AND CROSSING OVER IN DROSOPHILA MELANOGASTER

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THE conclusion that crossing over occurs invariably in association with the two-by-two pairing of homologous chromosomes is supported by two independent lines of evidence. First, cytological observations of chromosome behavior during pachytene of meiosis show that the homologous chromosomes, almost without exception, are regularly and intimately paired in a two-by-two fashion. Second, genetic studies of crossing over in a wide variety of plants and animals have demonstrated that whatever the mechanism, the complementary products of a crossover are formed without increasing or decreasing the number of loci per chromosome, a result consonant with two-by-two pairing. In short, most facts agree that the crossing over event occurs without alteration in the over-all continuity or integrity of the participating chromosomes.

Exceptions to regular pairing behavior in the form of non-homologous or "illegitimate" associations which if accompanied by crossing over would lead to aberrant chromosomes, have been recorded in several plants and animals. Cytological detection of non-homologous chromosome pairing have not infrequently been observed in plant material e.g., by MC CLINTOCK (1933) in maize, etc. By inference the spontaneous occurrence in Drosophila of long deficiencies, e.g., Notch-8 (MOHR 1919) and Notopleural (BRIDGES, SKOOG, and LI 1936) and extended tandem duplications, e.g., Bar (STURTEVANT 1925; BRIDGES 1936; MULLER, PROKOFYEVA-BELGOVSKAYA, and KOSSIKOV 1936), the Beadex-recessive mutants (GREEN 1953a,b) and the Star-duplication (LEWIS 1941) in *Drosophila melanogaster* occur as a result of crossing over between paired non-homologous chromosome segments. However, the data suggest that crossing over within paired non-homologous chromosome parts is, at best, rare and sporadic.

It is the purpose of this report to describe the regular occurrence of exceptional crossover products which apparently result from the crossing over within paired segments which appear to be, genetically speaking, non-homologous. The events to be described were uncovered as part of a study of pseudoallelism at the white (*w*) locus in *D. melanogaster* described in detail elsewhere (GREEN 1959).

## MATERIALS AND METHODS

As an aid in simplifying the presentation of the experimental results, the common laboratory mutants used have been listed in Table 1. Where special stocks were derived and used, they will be described in the text. The usual Drosophila culture methods utilizing a standard corn meal-Brewer's yeast-

TABLE 1

*Synopsis of mutants used in text. Symbols and map position generally after BRIDGES and BREHME (1944)*

| Mutant gene | Symbol | Map position |
|---|---|---|
| yellow body color | *y* | X–0.0 |
| yellow-2 body color | $y^2$ | allele of *y* |
| scute bristles | *sc* | X–0.0 |
| suppressor of apricot | *su-w*$^a$ | X–0.05 |
| Hairy wing | *Hw* | X–0.± |
| zeste eye color | *z* | X–1± |
| white eye color | *w* | X–1.5 |
| apricot | $w^a$ | pseudoallele of *w* |
| apricot-2 | $w^{a2}$ | allele of $w^a$ |
| apricot-3 | $w^{a3}$ | allele of $w^a$ |
| apricot-4 | $w^{a4}$ | allele of $w^a$ |
| coral | $w^{co}$ | allele of $w^a$ |
| colored | $w^{col}$ | pseudoallele of $w^a$ |
| split bristles | *spl* | X–3.0 |
| echinus eyes | *ec* | X–5.5 |
| singed-3 bristles | $sn^3$ | X–21 |
| miniature-2 wings | $m^2$ | X–36.1 |
| garnet-4 eye color | $g^4$ | X–44.4 |
| forked bristles | *f* | X–56.7 |
| Bar eye | *B* | X–57 |
| Curly wings | *Cy* | Inversions, chromosome 2 |
| Ultrabithorax-130 | $Ubx^{130}$ | Inversions, chromosome 3 |

molasses-agar medium were employed throughout. Flies were raised in a room whose controlled temperature fluctuated between 22–24°C.

In the genotype notations used herein the mutant gene designations as listed in Table 1 will be employed. Absence of a gene symbol means that the standard or wild type allele is present. Symbols are those found in BRIDGES and BREHME (1944).

## EXPERIMENTAL

*Exceptions occurring in association with crossing over:* In the study of pseudoallelism at the white locus, crossing over results were obtained showing that the mutants $w^a$ and $w^{a2}$ are pseudoalleles of the mutant $w^{ch}$. Both are localized to the left of $w^{ch}$. Experiments were therefore undertaken to determine whether $w^a$ and $w^{a2}$ occupied the same or separate loci. For this purpose females of the genotype $y^2$ *su-w*$^a$ $w^a$ *spl*/$w^{a2}$; *Cy*/+; *Ubx*/+ were obtained and crossed to *y w spl* $sn^3$ males. Among the progeny of this cross listed in Table 2, no wild type exceptions were obtained. However, five females all *w spl* in phenotype were found. Each female exception was separately progeny tested by crossing to *y w spl* $sn^3$ males. All females produced identical progeny consisting of females *y w spl* ($sn^3$) and *w spl* and males *y w spl* ($sn^3$) only. The sex ratio in each case was approximately 2 ♀ : 1 ♂. These results were interpreted to mean that the *w spl* chromosome was

TABLE 2

*The occurrence of white-eyed females exceptions in association with crossing over. All parental females heterozygous for* Cy *and* $Ubx^{130}$ *inversions unless otherwise noted and crossed to* y w spl $sn^3$ *males*

| Genotype parental females | Total white males | | Total male progeny |
|---|---|---|---|
| | *y* or $y^2$ | *spl* or *ec* | |
| $y^2$ *su-*$w^a$ $w^a$ *spl*/$w^a$ | 1 | 2 | 43,237 |
| *y* $w^{a2}$ *spl*/$w^{a2}$ | 9 | . | 95,062 |
| $y^2$ *su-*$w^a$ $w^a$ *spl*/$w^{a2}$ | . | 5 | 31,970 |
| $y^2$ *su-*$w^a$ $w^a$ *spl*/$w^{a2}$* | . | . | 45,411 |
| $y^2$ *sc* $w^a$ *ec*/$w^{a2}$ | . | 2 | 46,746 |
| $y^2$ *su-*$w^a$ $w^a$ *spl*/$w^{col}$ | . | . | 51,077 |
| *y ac* $w^{col}$ *spl*/$w^{a2}$ | 2 | . | 83,381 |
| $y^2$ *su-*$w^a$ $w^a$ *spl*/$w^{a3}$ | . | . | 55,965 |
| *y* $w^{a3}$ *spl*/$w^{a2}$ | . | 6 | 48,469 |
| $y^2$ *su-*$w^a$ $w^{a4}$ *spl*/$w^{a2}$ | . | . | 50,007 |
| *sc* $w^{co}$ *ec*/$w^{a2}$ | . | . | 44,919 |

* No autosomal inversions.

male lethal and the recovery of only exceptional females was not due to chance. In a repeat cross where autosomal inversions were omitted, no exceptions were found.

A parallel cross was made utilizing an $w^a$ chromosome of diverse origin. Here females $y^2$ *sc* $w^a$ *sc*/$w^{a2}$; *Cy*/+; *Ubx*/+ were crossed to $y^2$ *sc w ec* males. As noted in Table 2 white-eyed female exceptions carrying the *ec* marker were obtained. On progeny testing, the *w ec* chromosome similarly proved to be male lethal.

Subsequently a number of different crosses were made in which various *w* mutants were tested to both $w^a$ and $w^{a2}$. The results of crosses are listed in Table 2 and they show that associated with crossing over, additional white-eyed female exceptions were recovered from females $w^{a3}/w^{a2}$ but not $w^{a3}/w^a$; from $w^{col}/w^{a2}$ but not from $w^{col}/w^a$.

Finally tests among females homozygous $w^a$ and $w^{a2}$ were carried out using markers and heterozygous inversions as in other crosses and again, as noted in Table 2 associated with crossing over, white-eyed female exceptions were found. Two female exceptions were found in a stock $w^{a2}$; *Cy*; *Ubx/Xa*, one in an attached-X stock homozygous for $y^2$ *su-*$w^a$ $w^{a2}$.

In summarizing these results of Table 2 the following conclusions can be made. From a variety of crosses involving either the $w^a$ and/or the $w^{a2}$ mutants, white-eyed exceptional female progeny were found. The female exceptions always occur in association with crossing over. All exceptional chromosomes are male lethal. A complementary crossing over class was not detected since no other exceptions were found.

*The cytogenetic nature of the exceptional chromosomes:* Before a reasonable explanation for the origin of the exceptional types can be attempted, one pertinent question requires answering. Are all exceptional chromosomes identical or do they represent a variety of cytogenetic types? Since in each case the exceptional

chromosomes were male lethal and white-eyed, a tentative hypothesis was adopted that each is associated with a chromosome loss whose limits include in the very least the *w* locus. A cursory salivary gland chromosome examination of two of the exceptional types of independent origin kindly made by PROFESSOR E. B. LEWIS showed that each was associated with a deficiency of the X chromosome in the neighborhood of the *w* locus in which most of section 3A and all of section 3B are deleted, thereby supporting the hypothesis. Therefore the exceptional types will be referred to in all subsequent considerations as $w^{-r}$ or white deficiencies arising by recombination. Since a precise cytological description of each $w^{-r}$ exception appeared to be a laborious undertaking, analysis of the limits of the deficiencies was sought by genetic means. For such a program the *w* locus is uniquely suited since a number of duplications and deficiencies of this locus have been described.

As a first step in defining the limits of the $w^{-r}$ chromosomes, each was tested for coverage by four separate duplications of the *w* locus *viz.* Dp $w^{+51b7}$, Dp $w^{+51c20}$, Dp $w^{m59a}$ and Dp $w^{v,co}$. These will be referred to for simplicity as Dp-1, Dp-2, Dp-3 and Dp-4 respectively. In each duplication a segment of the X chromosome including the $w^+$ locus has been translocated to an autosome. The cytological limits of each are included in Table 3. Detailed descriptions are given by RATTY (1954) and BRIDGES and BREHME (1944). Stock cultures of each duplication were maintained in males by combining through appropriate crosses a white deficient ($w^-$) X chromosome carrying the marker *y* and a duplication covering the deficiency. These males were crossed to double-X females of phenotype *y w f*, thereby establishing the stock. Only males carrying both the deficiency and the duplication are viable in this stock; females may or may not carry the duplication.

After each of the $w^{-r}$ chromosomes was combined into a balanced lethal stock in which the balancer *In(1) sc*$^{8l}$ dl-49, *v f B* was used, tests for coverage were made as follows. Balanced females were crossed to duplication males and viable

TABLE 3

*Tests for coverage of two* w *deficiencies and representative* $w^{-r}$ *chromosomes by four duplications of the* w *locus. (Cytological limits of each duplication included in parentheses.) 0= duplication does not cover deficiency, += duplication covers deficiency*

| Deficiencies | Duplications Dp–1 (3C2–3D2) | Dp–2 (3C2–3C6) | Dp–3 (3B1–3E2) | Dp–4 (2C1–3C4) |
|---|---|---|---|---|
| $w^{-1}$ | 0 | 0 | + | + |
| $w^{-2}$ | + | + | + | + |
| $y^2\ w^{-r}$ (from $w^a/w^a$ ♀ ♀) | 0 | 0 | 0 | + |
| $w^{-r}$ *spl* (from $w^a/w^a$ ♀ ♀) | 0 | 0 | 0 | + |
| $w^{-r}$ *spl* (from $w^a/w^{a2}$ ♀ ♀) | 0 | 0 | 0 | + |
| $y^1\ w^{-r}$ (from $w^{a2}/w^{a2}$ ♀ ♀) | 0 | 0 | 0 | + |
| *y ac* $w^{-r}$ (from $w^{a2}/w^{col}$ ♀ ♀) | 0 | 0 | 0 | + |
| $w^{-r}$ *spl* (from $w^{a2}/w^{a3}$ ♀ ♀) | 0 | 0 | 0 | + |

exceptional males sought among the progeny. The following cross will illustrate the principle.

$$\text{♀ ♀ } w^r \, spl/In(1) \, sc^{S1} \text{ dl-49}, v \, f \, B \times \text{♂ ♂ } y \, w^r/\text{Dp-1}$$

If Dp-1 covers the $w^r$ loss, two types of $F_1$ males are expected, those being $v \, f \, B$ and $w^+$ *spl*. If Dp-1 fails to cover the $w^r$ chromosome only $v \, f \, B$ males emerge.

In Table 3 the results of tests for coverage by each of the four duplications with representative $w^r$ types and with two previously described independent $w$ deficiencies are listed. These results show that only Dp-4 covered all $w^r$ types, a fact which permits a tentative definition of the right-most limit of these deficiencies. Cytologically the $w$ locus has been delimited to salivary chromosome bands 3C1, 2 and 3. Since Dp-4 which extends just beyond 3C3 to 3C4 covers the $w^r$ deficiencies while the other three duplications which extend beyond 3C3 do not, it is concluded that the right-most limit of the $w^r$ deficiencies is band 3C4. In comparing the cytological lengths of Dp-3 and Dp-4 it will be noted that the former includes the region of the X chromosome from beyond the $w$ locus to bands 3B1,2 while the latter extends to band 2C1. Since Dp-3 does not cover the $w^r$ chromosomes whereas Dp-4 does, it follows that the chromosome losses associated with these deficiencies must extend beyond 3B1–2 to somewhere before 2C1. Thus at a minimum the whole of section 3B and part of 3C are missing in the derived deficiencies.

A more precise definition of the right-most limits of the $w^r$ deficiencies was obtained by testing them for allelism to a number of $w$ deficiencies of known cytological length. The following four deficiencies, the extent of their cytological losses following in parenthesis, were used as testers: $w^{258-45}$-(3C1); a $w$ deficiency derived from crossing over between *In(1)* $w^{m4}$ and *In(1)* $rst^3$-(3C2,3); $w^{258-48}$-(3B1–3C1) and $w^{258-11}$-(3A1,2–3C4). These deficiencies will be referred to simply as $w^{-1}$, $w^{-2}$, $w^{-3}$ and $w^{-4}$ respectively. All deficiencies were tested *inter se* and to representative $w^r$ chromosomes obtained from each of the crosses noted in Table 2. The method used in each case and typical results obtained are illustrated by the following crosses:

$$\text{♀ ♀ } w^{-2}/y \, Hw, In(1)\text{dl-49}, m^2 g^4 \times y \, w^{-1}/\text{Dp-1 ♂ ♂} \qquad \text{(cross 1)}$$

$$\text{♀ ♀ } w^r \, spl/In(1) sc^{S1}\text{dl-49}, v \, f \, B \times y \, w^{-1}/\text{Dp-1 ♂ ♂} \qquad \text{(cross 2)}$$

For each cross the phenotype, number and presumed genotype in parentheses of all female progeny are listed below.

| | | | |
|---|---|---|---|
| (cross 1) | ♀ ♀ *Hw* | 161 | ($y \, w^{-1}/y \, Hw \, In(1)$ dl-49 $m^2 g^4$ with or without Dp-1) |
| | ♀ ♀ + | 39 | ($w^{-2}/y \, w^{-1}$; Dp-1) |
| | ♀ ♀ *w* | 38 | ($w^{-2}/y \, w^{-1}$) |
| (cross 2) | ♀ ♀ *B*/+ | 98 | ($sc^{S1}$ dl-49 $v \, f \, B/y \, w^{-1}$; with or without Dp-1) |
| | ♀ ♀ + | 39 | ($w^r \, spl/y \, w^{-1}$; Dp-1) |

Of special interest in comparing these two crosses is the occurrence of white-eyed females among the progeny of cross (1) and not in cross (2). The genotype of the white-eyed females which, in comparison to their sisters were delayed in

eclosion by about two days, was interpreted as noted above to be a compound of both deficiencies. On progeny testing these white-eyed females to *B* males, the assumed genotype was confirmed since no males and only heterozygous *B* females were produced. This means that a deficiency for 3C1 compounded to a deficiency for 3C2,3 is viable, a situation not unlike that reported in maize by McCLINTOCK (1944) for the chromosome 9 losses *py* and *wd*. Since in cross (2) no white-eyed females emerged it is concluded that female zygotes compounded of the two deficiencies are lethal because they are, in part, homozygous for the loss of the same region.

From the results of the allelism tests listed in Table 4 the following conclusions are warranted. (1) Females compounded of 3C1/3C2,3 deficiencies are viable and white-eyed. (2) All $w^{-r}$ deficiencies are viable in compound with the 3C2,3 deficiency, none with those that include the loss of 3C1. (3) All $w^{-r}$ deficiencies are lethal when tested *inter se*. Therefore, it is concluded that all $w^{-r}$ deficiencies have one feature in common, the loss of band 3C1 and the presence of bands 3C2,3.

The evidence from the coverage by duplications placed the left-most limits of the $w^{-r}$ losses between salivary chromosome bands 2C1 and 3B1,2. On the basis of the elegant analysis of the zeste (*z*) locus (GANS 1953) it was possible to define with greater precision the limits of the $w^{-r}$ losses by determining whether *z*, localized to band 3A3, is or is not included. For this purpose GANS has provided

TABLE 4

*Allelism tests among white deficiencies and representative* w$^{-r}$ *chromosomes*

| | $w^{-1}$ | $w^{-2}$ | $w^{-3}$ | $w^{-4}$ | $\frac{\gamma^2\ w^{-r}}{(w^a/w^a)}$ | $\frac{w^{-r}\ spl}{(w^a/w^a)}$ | $\frac{w^{-r}\ spl}{(w^a/w^{a2})}$ | $\frac{\gamma\ w^{-r}}{(w^{a2}/w^{a2})}$ | $\frac{\gamma\ ac\ w^{-r}}{(w^{a2}/w^{co1})}$ | $\frac{w^{-r}}{(w^{a2}/w^{a3})}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| $w^{-1}$ | L | w | L | L | L | L | L | L | L | L |
| $w^{-2}$ | | L | w | L | w | w | w | w | w | w |
| $w^{-3}$ | | | L | L | L | L | L | L | L | L |
| $w^{-4}$ | | | | L | L | L | L | L | L | L |
| $\frac{\gamma^2\ w^{-r}}{(w^a/w^a)}$ | | | | | L | L | L | L | L | L |
| $\frac{w^{-r}\ spl}{(w^a/w^a)}$ | | | | | | L | L | L | L | L |
| $\frac{w^{-r}\ spl}{(w^{a2}/w^a)}$ | | | | | | | L | L | L | L |
| $\frac{\gamma\ w^{-r}}{(w^{a2}/w^{a2})}$ | | | | | | | | L | L | L |
| $\frac{\gamma\ ac\ w^{-r}}{(w^{a2}/w^{co1})}$ | | | | | | | | | L | L |
| $\frac{w^{-r}\ spl}{(w^{a2}/w^{a3})}$ | | | | | | | | | | L |

L = lethal, w = viable white-eyed females. See text for description of deficiencies and methods. For $w^{-r}$, *w* mutants of parental females follow in parentheses.

a simple, straight forward genetic test which utilizes a duplication designated by her as Dp (1–1) $z^4$. She found that the eye phenotype of females compounded of Dp (1–1) $z^4$ and a $w$ deficiency depends upon whether the $z^+$ locus is or is not included in the deficiency. For example, females Dp (1–1)$z^4/w^{258-11}$ (loss extending from 3A1 and therefore including $z^+$) are zeste in eye color, whereas females Dp (1–1)$z^4/w^{258-14}$ (loss extending from 3A4 and therefore not including $z^+$) are wild type in eye color. Accordingly representative $w^{-r}$ chromosomes derived from each of the crosses tabulated in Table 2 were tested to Dp (1–1)$z^4$ and the eye colors scored. Without exception females Dp (1–1)$z^4/w^{-r}$ proved to be wild type demonstrating that in each $w^{-r}$ chromosome the $z^+$ locus is not deleted.

Taking into consideration all tests, it can be concluded that at a maximum all $w^{-r}$ losses lack bands 3A4–3C1 inclusive.

In evaluating the known phenotypic interrelationship between the $z$ and $w$ loci and the nature of the $w^{-r}$ chromosomes described, two questions arose. Are the mutants $w^a$, $w^{a2}$, etc. necessary for the occurrence of the $w^{-r}$ chromosomes? Is the genetic state at the $z$ locus important in the derivation of the $w^{-r}$ deficiencies? Significant answers to these questions could, it was felt, provide a basis for interpreting the origin of the $w^{-r}$ losses.

Examination of the results in Table 2 shows that $w^{-r}$ losses were recovered from females possessing at least one X chromosome carrying either $w^a$ or $w^{a2}$. However, in at least two cases, parallel results were not obtained. From females $w^{a2}/w^{a3}$, $w^{-r}$ recombinants were obtained, while none was found among a comparable number of progeny of $w^a/w^{a3}$ females. Similar results were noted for females $w^{a2}/w^{col}$ and $w^a/w^{col}$. These facts suggested that it would be worthwhile to know whether the $w^{a2}$ and $w^a$ mutants are necessary for the origin of $w^{-r}$ chromosomes. In one test an apparent back mutation of $w^{a2}$ to wild type (designated $w^{a2+}$) was used. It arose as a single male $w^{a2+}$, $Ubx^{130}/+$ among the progeny of ♀♀ $w^{a2}/y^2$ *su-w*$^a$ $w^{a4}$ *spl*; *Cy*/+; $Ubx^{130}/+$ × ♂♂ *y w spl* $sn^3$. The $w^{a2+}$ chromosome was compounded in females with $w^a$ and $w^{-r}$ exceptions were sought among the progeny of the cross ♀♀ $w^{a2+}/y^2$ *su-w*$^a$ $w^a$ *spl*; *Cy*/+; *Ubx*/+ × *y w spl* $sn^3$ ♂♂.

The results listed in Table 5 show that in association with crossing over, $w^{-r}$ exceptions were found demonstrating that the presence of the mutant $w^{a2}$ is not prerequisite to the origin of the $w^{-r}$ deficiencies.

The requirement for the $w^a$ mutant was tested by using two independent partial back mutations of $w^a$ which differ from each other in phenotype. One back mutant, $w^{aM}$, made available through the kindness of MRS. J. C. MOSSIGE, arose in the so-called *Basc* chromosome and is intermediate between $w^a$ and + in phenotype. The second, $w^{a57i}$, arose as a single male in a $y^2$ *su-w*$^a$ $w^a$ *spl*; *Cy*; *Ubx/Xa* stock and is nearly wild type in appearance. Each back mutant was tested to $w^{a2}$ and the results listed in Table 5. These results show that while $w^{-r}$ deficiencies were recovered among the progeny of $w^{a2}/w^{aM}$ females, none were found among the progeny of $w^{a2}/w^{a57i}$ females. Since $w^{a57i}$ arose in a $w^a$ chromo-

TABLE 5

*Effect of substitutions at the* $w^{a2}$, $w^{a}$ *and* z *loci on the occurrence of* $w^{-r}$ *chromosomes. All parental females heterozygous for* Cy *and* $Ubx^{130}$ *inversions and crossed to* y w spl $sn^{3}$ *males*

| Genotype parental females | Total $w^{-r}$ females $y^{+}$ | *spl* | Total female progeny |
|---|---|---|---|
| $y^{2}$ *su-w*$^{a}$ $w^{a}$ *spl*/$w^{a2+}$ | 1 | 4 | 36,022 |
| $y^{2}$ *su-w*$^{a}$ $w^{aM}$ *spl*/$w^{a2}$ | 1 | 2 | 41,706 |
| $y^{2}$ *su-w*$^{a}$ $w^{a57i}$ *spl*/$w^{a2}$ | . | . | 49,197 |
| *sc z* $w^{a}$ *spl*/$w^{a2}$ | . | 4 | 34,644 |
| $y^{2}$ *su-w*$^{a}$ $w^{a}$ *spl*/*z* $w^{a2}$ | 1+1? | . | 42,503 |
| $y^{2}$ *su-w*$^{a}$ $w^{a}$ *spl*/$z^{+o}$ $w^{a2}$ | 1 | . | 29,736 |
| $y^{2}$ *su-w*$^{a}$ $w^{a}$ *spl*/$z^{+c}$ $w^{a2}$ | 1 | . | 45,927 |

? =♀ $y^{a}$ *w* in phenotype but sterile.

some which in combination with $w^{a2}$ produced numerous $w^{-r}$ losses, it appears that either $w^{a}$ or a related mutant is needed for the $w^{-r}$ losses to occur.

To test the *z* locus, the mutant *z* was introduced into both $w^{a}$ and $w^{a2}$ bearing X chromosomes. These were then tested, in the presence of the usual heterozygous *Cy* and $Ubx^{130}$ inversions, to $w^{a2}$ and $w^{a}$ respectively. The results listed in Table 5 show that the introduction of the mutant *z* had contrary effects in the two X chromosomes. In the $w^{a}$ chromosome the *z* mutant apparently had no effect in altering the production of $w^{-r}$ deficiencies, for these losses occurred in a frequency and with a marker distribution precisely as though no *z* mutant was present. On the other hand the presence of the *z* mutant in the $w^{a2}$ chromosome had a significant effect, for only one (possibly two) $w^{-r}$ chromosome was recovered and it was in association with the *y* rather than the *spl* marker gene. That this substitution of *z* into the $w^{a2}$ chromosome was not a chance observation was demonstrated in another way. In place of *z* in the $w^{a2}$ chromosome the $z^{+}$ loci from the Canton-S and Oregon-R wild type stocks were substituted. These chromosomes, designated $z^{+c}$ $w^{a2}$ and $z^{+o}$ $w^{a2}$, were separately tested to $w^{a}$ and $w^{-r}$ losses were sought. The results of these tests, enumerated in Table 5, show that in effect the introduction of $z^{+c}$ and $z^{+o}$ in the $w^{a2}$ chromosome paralleled precisely the introduction of *z*. In both cases there was a reduction in the frequency of $w^{-r}$ deficiencies and a switch in the marker association. These facts mean that the genetic state of the *z* locus in the $w^{a2}$ chromosome has a paramount role in the origin of the $w^{-r}$ losses.

What explanation can be offered for the origin of the $w^{-r}$ deficiencies? Obviously crossing over between regularly paired chromosomes provides no satisfactory explanation. By postulating, however, that single crossing over occurs concomitant to a pairing association between noncontiguous segments of homologous X chromosomes, a satisfactory mechanism for the origin of the $w^{-r}$ losses is provided. Since the $w^{-r}$ losses include at a maximum the salivary chromosome bands 3A4 to 3C1, pairing and crossing over must occur so as to yield such a loss. The simplest pairing scheme which would yield a $w^{-r}$ loss would involve the

"illegitimate" pairing of band 3A3 (the *z* locus) with 3C1 of the homologous chromosome as follows:

3A1,2,3,4, . . . . 9 3B1,2 . . . . 3C1,2,3
. . . . . 3A1,2,3,4 . . . . 9 3B1,2 . . . 3C1,2,3

If within the paired region a crossover occurs between 3C1.2 of one chromosome and 3A3,4 of the homologue, the two products of the crossover event will be: (1) a chromosome in which the region extending from 3A4 to 3C1 has been lost and whose salivary chromosome band complement can be written as 3A1,2,3 C1,3,4 and (2) a chromosome in which the deleted region of (1) has been duplicated in tandem and which can be designated as 3A1,2,3,4 . . . . B1,2 . . . C1 A4 . . . B1,2 . . . C1,2,3. The deletion coincides with the limits of $w^{-r}$ but the duplication has not been uncovered.

Assuming the pairing and crossing over scheme described does in fact occur, the question may be raised as to why the duplication has not been recovered. Since the $w^{-r}$ chromosomes represent the only detectable exceptions, it is clear that the duplication, if it occurs, has no distinctive phenotype with which it can be associated. This is in a sense surprising since the $w^{-r}$ chromosomes behave phenotypically as losses of the *w* locus, and it would follow that a duplication equivalent to $w^{-r}$ would act phenotypically as a duplication of the *w* locus. From this line of reasoning it would follow that since the loss derived e.g., from $w^a/w^{a2}$ females behaves as a *w* locus loss, the reciprocal would be a duplication of the *w* locus and be phenotypically distinctive from either $w^a$ or $w^{a2}$.

There are a number of reasons which suggest that while the scheme proposed for the origin of the duplication is plausible, the anticipated phenotypic effects are not. The available facts support the idea that genic mutants associated with the *w* locus are localized to the doublet 3C2,3, and that duplications and deficiencies of band 3C1 do not involve the *w* locus *per se*. The recombination analysis of the white mutants shows that four loci are indicated. Mutants representing each of the loci, including all four at the left-most locus, have been tested for coverage by Dp-1 and Dp-2 both of which include the doublet 3C2,3 and lack band 3C1. These duplications cover all mutants tested. Tests with *w* deficiencies considered earlier show that Dp-1 and Dp-2 cover the deficiency for the 3C2,3 doublet but not the deficiency for band 3C1. Two conclusions follow from these data: first, the *w* gene mutations are localized to the doublet 3C2,3; second, since a duplication of 3C2,3 fails to cover an alteration associated with 3C1, it is not unexpected that a duplication of 3C1 will not affect phenotypes associated with mutations in 3C2,3. Therefore it is not unreasonable that the duplication reciprocal to the $w^{-r}$ losses is without phenotypic effect on mutants of the *w* loci.

Two additional exceptions were recovered among the progeny of homozygous $w^a$ females which differed from the $w^{-r}$ losses. From the homozygous $w^a$ females listed in Table 1, a single $y^2$ *spl*$^+$ male was recovered whose eye color was distinctly darker than that associated with the *su-*$w^a$ $w^a$ genotype. A single male of similar phenotype was found in a stock of genotype $y^2$ *su-*$w^a$ $w^a$ *spl*; *Cy*; *Ubx/Xa*

and is assumed to have arisen by crossing over. Both exceptional types proved to be identical and will be considered together. Cytological examination kindly provided by PROFESSOR E. B. LEWIS showed that both exceptions are associated with tandem duplications involving regions 3B and 3C of the X chromosomes. These duplications, which will be called Dp $w^a$, are somewhat shorter than those found by LEWIS (1956) to be associated with the white locus. Genetic information substantiated the tandem duplication nature of the exceptions and showed that in all probability the $w^a$ mutant together with the other $w$ loci is duplicated. In one test of females *y ac* $w^+$ *spl*/Dp $w^a$, 34 *y ac* $w^a$ and 4 $w^a$ *spl* males were recovered among 11,915 male progeny. These results fit the assumption that in Dp $w^a$ the duplicated region to the left of the $w^a$ locus, e.g., section 3B, is genetically much longer than the duplicated region to the right of the $w^a$ locus. If the duplicated left segment is represented for simplicity by the letters *a,b,c* and the duplicated right segment by the letter *d*, then the duplication can be written as *a,b,c,*$w^a$*,d*, *a,b,c,*$w^a$*,d*; the normal chromosome as *a,b,c,*$w^+$*,d*.

In female *y ac* $w^+$ *spl*/Dp $w^a$ when pairing occurs as:

$$\begin{array}{ll} & a,b,c,w^a,\ d,a,b,c,w^a,d,e,f \\ y\ ac & a,b,c,w^+,d \ldots\ldots\ldots\ldots spl \end{array}$$

a crossover between $w^a$ and *d* in the paired segment will ultimately yield a male $w^a$ *spl*. Alternatively when the pairing occurs as:

$$\begin{array}{l} a,b,c,w^a,d\ a,b,c,w^a,d \\ y\ ac \ldots\ldots a,b,c,w^+,d \ldots\ldots spl \end{array}$$

crossovers to the left of $w^a$ and $w^+$ in the paired segment, e.g. between *a,b* etc., will produce *y ac* $w^a$ males. On the assumption that the two pairing schemes occur with equal frequency, the recovery of 34 *y ac* $w^a$ males compared to four $w^a$ *spl* males demonstrates that genetically the duplicated chromosome segment to the left of $w^a$ is greater than that to the right of $w^a$.

Since it is conceivable that the crossover product complementary to the $w^-$ loss is a duplication carrying both $w^a$ and $w^{a2}$, the $w^{a2}$ mutant was substituted for one $w^a$ in Dp $w^a$ in order to assess the phenotype of this combination. This substitution was rather laboriously carried out by constituting females of the genotype *y ac* Dp $w^a$ *spl*/$w^{a2}$ and selecting *y ac* non-$w^{a2}$ male progeny for further testing. Such males, all phenotypically Dp $w^a$, could arise either by a crossover inserting $w^{a2}$ into the right segment of the duplication or as a crossover outside the duplication between $w^a$ and *spl*. The latter represents an unaltered Dp $w^a$. A number of crossovers were progeny tested by crossing to *Cy*; *Ubx/Xa* females, selecting the $F_1$ *Cy*; *Ubx* females and scoring the $F_2$ males. If the *y ac* males carried the unaltered Dp $w^a$ then only $y^+$ $ac^+$ $w^a$ crossover $F_2$ males should occur. On the other hand, if $w^{a2}$ had been inserted into Dp $w^a$, then $F_2$ $y^+$ $ac^+$ $w^{a2}$ crossover males should occur. Two cases of the latter type were found. This means that the Dp with $w^a$ in its left and $w^{a2}$ in its right segment is phenotypically equivalent to Dp $w^a$. Since males of this phenotype are readily separable from

either $w^a$ or $w^{a2}$ males, they would hardly escape detection if they occurred as the reciprocal to the $w^{-r}$ losses. Their absence means that the duplication carrying both $w^a$ and $w^{a2}$ cannot represent the crossover concomitant to the occurrence of $w^{-r}$.

How frequently does the "illegitimate" pairing occur in $w^a/w^{a2}$ females? Before an estimate can be attempted, information is needed on the crossing over frequencies in the interval *y* or *sc-z-w* or from the distal end of the X chromosome to the *w* locus. GANS (1953) localized *z* at a point genetically equidistant between *sc* and $w^{co}$. In studying the genetic interactions between *z* and several *w* mutants (GREEN 1959) an estimate was made of the genetic distance for the interval *sc-z-w* in the following way. For each of 35 independent *w* mutants, males *sc z w* were sought among the progeny of the cross ♀♀ *sc z ec ct/w* × *sc z ec ct* ♂. $F_1$ *sc w* males were progeny tested for the presence of *z* by crossing to homozygous *sc z ec ct* females. After progeny testing 214 males, 139 or 65 percent proved to be *sc z w*, 75 or 35 percent *sc* $z^+$ *w*. This means that for the genetic interval *sc-w*, 35 percent of the genetic distance separates *sc* and *z* while remaining distance separates *z* and *w*. Comparable results were obtained by measuring the genetic interval *y-z-*$w^a$ as influenced by the heterozygous inversions *Cy* and $Ubx^{130}$. For this purpose females *y z ec ct/*$w^a$; *Cy/+*; *Ubx/+* were crossed to *y z* males. Since the eye color of *z* $w^a$ males is distinctly more dilute than that of $w^a$ males, and thereby permits a perfect separation, crossing over was estimated by enumerating males $w^a$ (noncrossover), *y* $w^a$ (crossover between *y* and *z*) and *y z* $w^a$ (crossover between *z* and $w^a$). Among 1661 males, 41 or 2.47 percent were *y* $w^a$ and 57 or 3.43 percent were *y z* $w^a$ or the *y-z* and *z-*$w^a$ intervals constitute 42 percent and 58 percent respectively of the entire interval.

It is of interest to note here the relationship between salivary gland chromosome map distance and genetic distance for the loci *y-z-*$w^a$. These mutants have been localized to bands 1A5–8, 3A3 and 3C2,3 respectively. In terms of the number of salivary gland chromosome bands, the *y* and *z* loci are separated by a minimum of 65 bands whereas the *z* and $w^a$ loci are separated by only ten bands. Thus the salivary chromosome distance for this region conveys a misleading view of the relationship between cytological and genetical length, a fact already pointed out by others, and the region with the fewer bands and gene loci has the greater genetic distance.

An estimate of the frequency of non-homologous pairing can be obtained from the following considerations. The frequency of $w^{-r}$ exceptions was found to be approximately 1/10,000 females. Since the reciprocal crossover is presumed to occur although was undetected, the total crossover frequency is 1/5000 or 0.02 percent. For simplicity, it will be assumed that (1) crossing over occurs between bands, and (2) the frequency of crossing over between any pair of contiguous bands is equal whether or not pairing is two-by-two. For the interval 3A3–3C2 crossing over can occur between any one of twelve band pairs. On the basis of the crossing over frequency between *z-*$w^a$ as influenced by autosomal inversions, the frequency of crossing over between a band pair in this interval is

about 0.3 percent. Granting the postulate that the exceptions occur when a crossover occurs between bands 3C1,2 of one chromatid and 3A3,4 of the nonsister chromatid, it follows that 1/15 (0.02/0.3) of all the crossovers which occur between each band pair take place when 3C1,2 is so paired with 3A3,4 as to produce either the $w^{-r}$ loss or the undetected duplication. While it is conceded that the assumptions underlying this estimate are unverifiable and probably not realistic, they do strongly suggest that the frequency of non-two-by-two pairing must be high indeed, higher than reflected by the frequency of exceptions recovered.

## DISCUSSION

To explain the occurrence of the $w^{-r}$ chromosomes the following scheme was proposed. First, during meiosis pairing occurs between bands 3C1,2 of one chromatid and 3A3,4 of the nonsister chromatid. Second, a crossover occurs within the paired region yielding a deficiency extending from bands 3A4 to 3C1 inclusive and a reciprocal tandem duplication of the equivalent chromosome segment. On the basis of the crossing over marker genes recovered in association with the $w^{-r}$ chromosomes, two pairing situations have been encountered. Those $w^{-r}$ chromosomes derived, for example, from female $y^2$ *su-w*$^a$ $w^a$ *spl*/$w^{a2}$ carried the *spl* marker. Their occurrence is explained by assuming that pairing occurs between 3C1,2 of the $w^a$ chromosome and 3A3,4 of the $w^{a2}$ chromosome as follows.

$$\begin{array}{r} y^2 \ldots\ldots 3A3,4 \ldots . C1,2,3 \ldots\ldots . spl \\ 3A3,4 \ldots . C1,2,3 \end{array}$$

The appropriate crossover produces a deficiency carrying *spl*.

Alternatively those $w^{-r}$ chromosomes recovered, for example, from females $y$ $w^{a2}$ *spl*/$w^{a2}$ carried the $y$ marker. Here pairing is assumed to occur according to the following scheme.

$$\begin{array}{lll} y & \ldots 3A3,4 \ldots\ldots C1,2,3 & spl \\ & 3A3,4 \ldots C1,2,3 & \end{array}$$

The appropriate crossover produces a deficiency carrying the $y$ marker.

The type of "illegitimate" meiotic pairing associations invoked here is not without precedent especially if one extrapolates from comparable pairing associations observed in the salivary gland chromosomes of Drosophila and Sciara. BRIDGES (1935, 1938) recorded in *D. melanogaster* the occurrence in the salivary gland chromosomes regions of "confused clumping due to synapsis of homologous bands along the chromosomes." These he attributed to pairing of "repeats" or putative duplicate gene loci. METZ (1947) likewise has been impressed with the cytological evidence for the occurrence of "repeats" in Sciara and has figured the occurrence in *S. ocellaris* a triple "repeat" in the salivary gland X chromosome which imposed concurrent synapsis upon three nonadjacent chromosome regions.

In BRIDGES' view the pairing associations of "repeats" could provide a means for obtaining duplications and deficiencies of the types described here. This ex-

planation is not unlike that submitted by CATCHESIDE (1947) to explain the occurrence of a duplication and deficiency in *Oenothera blandina*.

While there is no convincing evidence for the occurrence of duplicate genes in Drosophila, data are nevertheless available to show that the *z* locus (band 3A3) and band 3C1 manifest an intimate genetic relationship. This despite the absence of a clear-cut demonstration that 3A3 and 3C1 are cytologically "repeat" loci. GANS (1953) clearly demonstrated experimentally that genotypic *z* males are wild type in phenotype unless bands 3C1,2 and 3 are duplicated. In duplication males the mutant *z* eye color develops. Additional evidence shows that these genetic affinities can be more specifically attributed to band 3A3 and 3C1. As described above, the loss of band 3C1 inhibits the phenotypic effects of DP (1–1) $z^4$. In addition females homozygous for the mutant *z* but heterozygous for a deficiency which includes band 3C1 but not 3C2,3 are wild type in phenotype (GREEN, unpublished observations). While these facts do not establish an homology between 3A3 and 3C1, they do support the interpretation of their fundamental genetic interdependence. As such the occurrence of pairing between 3A3 and 3C1 is not a completely surprising event.

Needless to say a number of experimental facts described herein defy an unequivocal explanation at this time. Data were presented which show that the $z^+$ locus in the $w^{a2}$ X chromosome has a marked pairing affinity with 3C1 in the $w^a$ and $w^{a3}$ carrying chromosomes. This is supported by the fact that this pairing configuration is reversed when the mutant *z* or $z^+$ loci from Canton-S and Oregon-R wild type stocks are substituted for the $z^+$ locus of the $w^{a2}$ stock. On the other hand the $z^+$ locus of the $w^{a2}$ stock appears to have a marked pairing impotency for band 3C1 carried by the *y ac* $w^{col}$ *spl* and *y* $w^{a2}$ *spl* chromosomes. Does this mean that the 3A3 and 3C1 bands allelic differences occur which manifest varying pairing affinities toward one another? Clearly this is a question of fundamental nature bearing upon the factors and forces which bring chromosomes together in what is recognized as meiotic pairing. The last word on this subject has yet to be written. In fact critical data relevant to this phenomenon are meager indeed and no claim is made that data submitted here add significantly to this subject.

In a general way the occurrence of non-homologous pairing and crossing over as described herein has a bearing on the mechanism of crossing over. It is difficult to reconcile the regular occurrence of the $w^{-r}$ and Dp $w^a$ chromosomes to a crossover event which demands that two nonsister chromatids be broken at dissimilar positions simultaneously followed by rejoining to produce the proper crossover products. Seemingly too many coincidental events are required to account for the crossover event. Perhaps the occurrence of deficiencies and duplications finds a better explanation in terms of a crossing over event which involves errors in chromatid reduplication imposed by non-homologous pairing. Such errors in reduplication would be consistent with the described copy-choice mechanism of crossing over.

SUMMARY

The regular occurrence in association with crossing over of a deficiency ($w^{-r}$) extending from bands 3A4–3C1 of the X chromosome of *D. melanogaster* is described. The occurrence of two independent identical tandem duplications associated in part with the white locus is also described.

A detailed analysis of the genetic conditions which influence the occurrence of the $w^{-r}$ chromosomes has been presented. Pairing of bands 3A3 and 3C1 followed by crossing over represents the simplest explanation for the origin of the $w^{-r}$ chromosomes.

The genetic relationships between bands 3A3 and 3C1 are discussed together with a brief discussion of the problem of crossing over.

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# A STUDY OF THE RELATIONSHIP BETWEEN KARYOTYPE AND PHENOTYPE IN CLONED LINES OF STRAIN HeLa[1]

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IT has been shown repeatedly by various workers (HAUSCHKA and LEVAN 1958; HAUSCHKA 1958; CHU and GILES 1958; CHU *et al.* 1958; BAYREUTHER and KLEIN 1958) that cloned lines of tumor cells with distinct physiological or morphological properties frequently differ from one another in observable characteristics of their karyotypes. The obvious question arises: are the distinct physiological or morphological properties of the various cell lines caused by the differences in the karyotypes, or, are the latter only an additional expression of the great variability of tumor cells? Most workers in this field consider the present evidence insufficient to decide the question in either way. The purpose of this paper is to present further data bearing on the same question. As will be seen, the data favor strongly the assumption of a causal relationship between karyotype and phenotype in a tissue culture line of human neoplastic cells, the strain HeLa (GEY *et al.* 1952).

Several factors may be held responsible for the difficulties encountered in a study of the relationship between karyotype and phenotype in tumor cell populations. One of the factors is the great frequency with which the karyotype changes in populations carrying aneuploid chromosome constitutions. In strain HeLa, for example, one out of a hundred mitoses on the average is abnormal (CHU and GILES 1958). Since most of these abnormal mitoses lead to an unequal distribution of the chromosomes to the daughter cells, new karyotypes are formed at a high frequency. Cloned cell populations contain therefore after a short period of growth a number of variant karyotypes. It was found possible to overcome this difficulty by studying the karyotypes of cloned populations within one to two months after the isolation from the single cell.

Another difficulty in this study lies in the great variety of karyotypes that arise as a consequence of the large number of chromosomes and their more or less random reassortment during abnormal mitoses. Due to the great variety of possible chromosome combinations, many different karyotypes may be expected to lead to phenotypes indistinguishable in respect to the mutant character studied. This situation excludes, therefore, *a priori*, the possibility to find a one-to-one relationship between karyotype and phenotype, which would be the most direct evidence of a causal relationship between karyotype and phenotype.

It is, however, possible to look for a partial solution of the problem by studying the relationship between karyotype and phenotype in one direction only, that is,

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by studying the karyotypes which correspond to given phenotypes. The possible results can be examined in the light of the following two hypotheses:

*Hypothesis 1.* The phenotype is not determined by the karyotype; any karyotype can correspond to a given phenotype.

*Hypothesis 2.* The phenotype is determined by the karyotype. Different phenotypes have, in general, different karyotypes.

Under hypothesis 1, each cell has, independent of its karyotype, the same probability to mutate to a new phenotype. The karyotype of most (if not all) variants should therefore fall into the same karyotype classes most frequently represented in the parental population. Under hypothesis 2, on the other hand, where each change in phenotype is caused by a karyotype change, the karyotypes of the variants should differ from those of the parental line. Hypothesis 2 predicts furthermore that karyotypes which have been selected for and which are maintained under strong selection pressure should vary less than karyotypes grown under nonselective conditions. In fact, the selection pressure would select against most of the new karyotypes arising from abnormal mitoses since most of these would not give rise to adaptive phenotypes.

The data to be presented give support to both predictions of hypothesis 2.

## MATERIAL AND METHODS

*Cell line*: The two clones, *St1* and *F8*, of strain HeLa used throughout this work were isolated from the clonal line S3 (PUCK and MARCUS 1956), kindly made available to us by DR. PUCK. The method of isolation and the properties of the two clones have been described previously (VOGT 1958).

*Culturing conditions:* Unless otherwise stated, all cell cultures were grown in 60 mm petri dishes in EAGLE's medium (1955), containing four times as much amino acids and vitamins and $4 \times 10^{-5}$M inositol (EAGLE *et al.* 1956), supplemented with 15 percent human and 15 percent horse serum. The cultures were incubated at 37°C in an incubator containing a well humidified five percent $CO_2$-air mixture. Single-cell platings for the counting of colony formers or for the isolation of clones were done according to the method described by PUCK *et al.* (1956). The aminopterin (4-amino-pteroylglutamic acid) used in this work was kindly supplied by Lederle Laboratories.

*Staining and counting of chromosomes:* For chromosomal studies, cells grown on coverslips were treated for three to eighteen hours with a $10^{-7}$M colchicine solution, exposed 2–10 minutes to a hypotonic solution, air-dried, fixed with methanol, stained with Feulgen and embedded in euparal. Each cell selected for counting was photographed with a 97× phase-contrast oil immersion objective and a 10× eyepiece. The position of each cell on the slide was recorded by using the calibration of the microscope stage. To minimize counting errors, special care was taken in choosing only cells with sharp outlines and widely spread chromosomes. The chromosomes were counted from seven fold enlargements of the negatives; each enlargement was then checked for details with the original preparation. Cases in which unresolvable ambiguities were encountered were

discarded. Cells with higher degrees of polyploidization were omitted from the counts, since they comprised only a small percent of the total population.

## RESULTS

A number of variants were obtained from two recently cloned lines of strain HeLa, S3, and the karyotypes of the parental and of the variant lines were compared. Two different methods for obtaining the variants were used. One method consisted in the use of selective agents, such as drugs or suboptimal growth media; variants capable of good growth in the presence of the drug or the suboptimal growth medium were selected for. The second method consisted in the isolation of spontaneous morphological variants which had accumulated in the cloned lines after a cultivation period of 15–19 months.

In the classification of a karyotype, two parameters have been used: the total number of chromosomes counted per cell, and the number of chromosomes corresponding to the three largest (L) metacentric chromosome pairs of the human idiogram. Although this is a limited characterization of the karyotype, it proved to be adequate, since it revealed karyotype differences in all cases in which the phenotype had changed.

*Karyotype distributions of the parental lines:* The origin of the two parental lines, *St1*, and *F8*, has been described previously (Vogt 1958). At the time of isolation the cells of clone *St1* were polygonal in shape and formed tightly packed colonies in single-cell platings. The cell line was characterized by a high sensitivity to poliovirus. The distribution of chromosome numbers counted over a culturing period of four to 14 months since the isolation of the line is shown in Figure 1. The two most frequent karyotypes present in the line after four to five months of cultivation were a karyotype with 78 (12 large) chromosomes, and a karyotype with 77 (11 large) chromosomes. After a culturing period of nine months, a new karyotype of 76 (12 large) chromosomes was found in 23 percent of the cells. The proportion of cells carrying the new karyotype increased with further cultivation. Parallel with the shift in the chromosome mode of the population, a change in the phenotypic appearance of the cell line was noticed. An increasing proportion of cells showed a phenotype intermediate (I) in its morphology between a polygonal (P) and a fusiform (F) cell type.

The second parental line, designated as *F8*, was isolated from the survivors of a S3 population that had been repeatedly exposed to poliovirus. Its cells were, at the time of isolation, fusiform in shape. Due to their tendency to migrate, they formed colonies of loose texture in single-cell platings. Cells of the *F8* line were 10–15 times more resistant to poliovirus than the cells of the *St1* line (Vogt 1958). A karyotype with 70 (nine large) chromosomes remained the most frequent karyotype over a culture period of six to 13 months (Figure 2).

*Influence of suboptimal growth conditions on the distribution of karyotypes:* Parallel to the passages in standard medium, which contains 15 percent human and 15 percent horse serum, parallel cultures of both parental lines were grown for seven to eight months in media containing as sole source of serum either ten

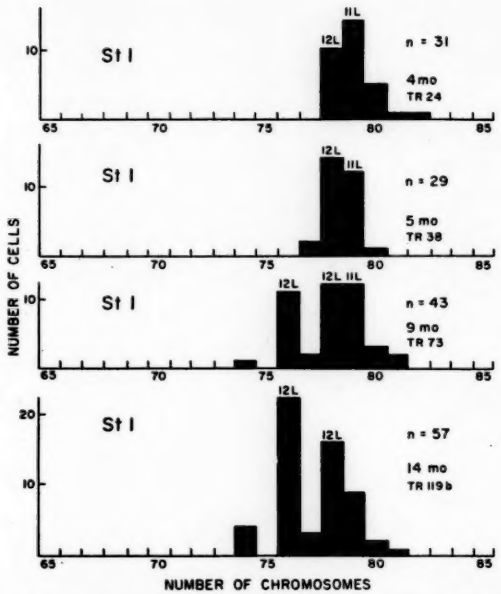


FIGURE 1.—Distribution of chromosome numbers in the parental *St1* clone after four to 14 months of growth in standard medium. The numbers above the columns indicate the numbers of large chromosomes present in each karyotype. The number of months refers to the time since the isolation of the single cell.

percent horse serum (*F8* line), or ten percent calf serum (*St1* line). In both media, a certain proportion of the cells died at the early passages, indicating that the cell populations were not well adapted to these media. At later passages, no cell death was observed, and the adapted lines had a growth rate similar to that of the control cultures grown in standard medium. Chromosome counts of the *F8* line after its growth in ten percent horse serum medium are given in Figure 2. A new karyotype with 72 (nine large) chromosomes, which was not present in the parental population, was found in approximately half of the cells after four months (tr 115), and in 28 out of 29 cells after eight months (tr 148) of growth in horse serum medium. The substitution of the parental karyotypes by a single new karyotype suggests a high selective advantage for the cells carrying this karyotype. The stability of the new karyotype over a culturing period of several months in the selective medium—a stability usually not encountered if cells are grown in standard medium—agrees well with the prediction of hypothesis 2. Single-cell platings of the horse-serum-adapted line in standard medium showed that the fusiform (F) phenotype of the original *F8* line had been replaced by an intermediate (I) phenotype. In addition, the resistance of the new line to poliomyelitis virus had decreased to one fifth of that of the original *F8* line.

The chromosome numbers obtained after seven months cultivation of a *St1* culture in ten percent calf serum are shown in Figure 3 (tr 40). Only two karyotypes were observed: a karyotype with 77 (ten large) chromosomes and a karyotype with 78 (ten large) chromosomes, respectively. Neither of the two karyotypes corresponded to any of the three most frequent classes found in the control parental population (Figure 1).

The analysis of cloned populations derived from the calf serum-adapted line showed the presence, in roughly equal proportions, of two cell types of markedly different colonial morphology. The cells of one type adhered strongly to the glass and formed colonies which were less compact than those of the original *St1* line. The cells of the second type had a small affinity to the glass and tended to pile up

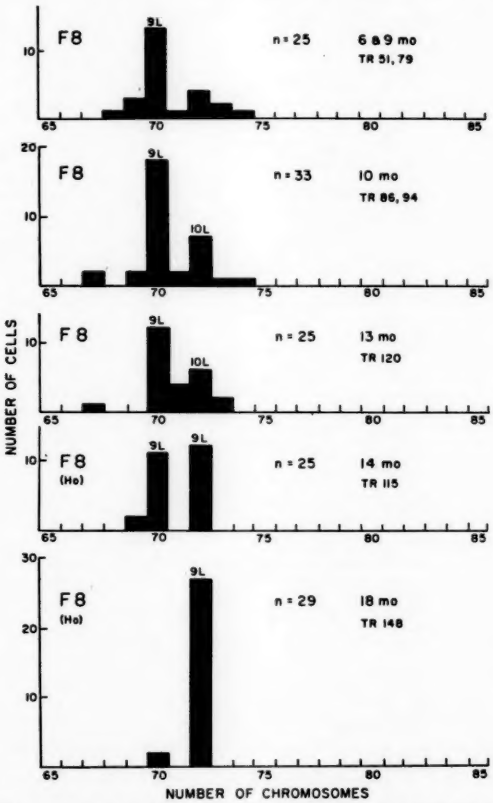


FIGURE 2.—Distribution of chromosome numbers in the parental *F8* clone. The upper three figures show the distribution after six to 13 months of growth in standard medium, the lower two figures after ten months in standard medium and four months (tr 115) or eight months (tr 148) of growth in horse serum medium.

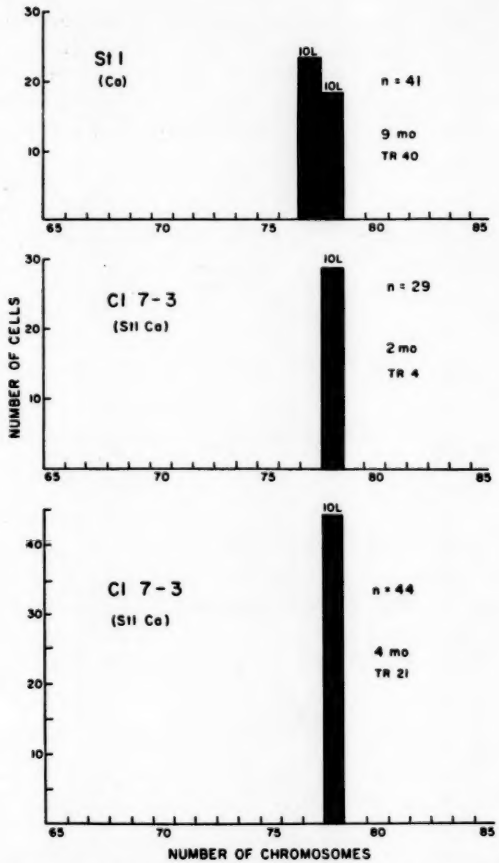


FIGURE 3.—Upper figure: chromosome numbers in the *St1* (Ca) line after two months of growth in standard medium and seven months in calf serum medium. Lower figures: chromosome numbers in clone *C1 7–3* after two and four months of growth in calf serum medium.

inside each colony. A clone of the second type was isolated and has now been grown for four months in the selective calf serum medium. In two samples of cells from this clone, examined two and four months after the isolation of the clone, a single karyotype was found, which corresponded to one of the two karyotypes present in the uncloned (adapted) population (tr 40, Figure 3). The stability of the karyotype over a culturing period of four months in selective medium is again best explained by assuming a strong selective advantage of this karyotype. Experiments have recently been started in which independent cultures of the calf serum-adapted clone are grown in parallel in standard, nonselective

medium, and in the selective calf serum medium. The comparison of the rates at which new karyotypes accumulate under both conditions will provide a more direct test of the correctness of the interpretation.

*Karyotype distributions in three cell populations with increased resistance to aminopterin:* The sensitivity to aminopterin (Am) was measured by plating aliquots of a given cell suspension into medium containing various concentrations of aminopterin, and counting the number of cell colonies after ten to 14 days of incubation. Fresh drug-containing medium was added to the cultures on the fourth, seventh and tenth day after the plating. The results of such experiments are given in Table 1. As may be seen from the table, a concentration of 0.0064 micrograms of aminopterin per milliliter suppresses completely colony formation in the parental *St1* line. A concentration of 0.004 μg/ml is only slightly inhibitory, since the number of colonies counted corresponded to 70 percent of the number of colonies found in the absence of the drug. In repeated platings of $2 \times 10^4$ *St1* cells per plate over a culturing period of 14 months, no colony formation was ever obtained at a concentration of 0.0064 μg Am/ml. The results in Table 2 show that

TABLE 1

*Survival, as colony formers, of* St1 *cells and of cells from three different aminopterin-resistant cell populations*

| Cell line | Aminopterin concentration in μg/ml | No. of cells plated per plate | No. of colonies counted per plate | Relative cell survival |
|---|---|---|---|---|
| *St1* | .... | 520 | 213,316 | 1.0* |
| tr 60 | 0.0040 | 520 | 179,198 | 0.71 |
| | 0.0046 | 520 | 156,121 | 0.52 |
| | 0.0054 | 520 | 48,37 | 0.16 |
| | 0.0064 | 26,000 | 0,0 | <0.00008 |
| | 0.0064 | 5,200 | 0,0 | ... |
| | 0.0080 | 26,000 | 0,0 | <0.00008 |
| | 0.0080 | 5,200 | 0,0 | ... |
| *Am 16–6* | .... | 480 | 297,286 | 1.0* |
| (aminopterin | 0.0046 | 480 | 127,229 | 0.62 |
| independent) | 0.0054 | 480 | 112,215 | 0.56 |
| | 0.0064 | 480 | 16,11 | 0.05 |
| | 0.0080 | 480 | 7,10 | 0.03 |
| *Am 36* | ... | 670 | 212,256 | 1.0* |
| (aminopterin | 0.032 | 670 | 238,189 | 0.91 |
| independent) | 0.064 | 670 | 112,144 | 0.55 |
| | 0.128 | 2,010 | 121,135 | 0.18 |
| *Am 32* | ... | 510 | 5,7 | 0.03 |
| (aminopterin | 0.016 | 510 | 190,246 | 1.0† |
| dependent) | 0.032 | 510 | 92,114 | 0.47 |
| | 0.064 | 510 | 64,73 | 0.32 |

* The survival is referred to the number of colonies counted in the absence of the drug.
† Since this cell line is aminopterin dependent, the survival is referred to the number of colonies counted at an aminopterin concentration of 0.016 μg/ml.

TABLE 2

*Plating efficiency of Am-resistant cells (line* Am 36*) in presence of Am-sensitive cells (line* St1*)*

| Aminopterin concentration in μg/ml | No. of cells plated per plate | | No. of colonies counted per plate | Efficiency of plating |
|---|---|---|---|---|
| | Am-sensitive cells | Am-resistant cells | | |
| 0.0064 | 0 | 500 | 220, 243 | 0.46 |
| 0.0064 | $5 \times 10^4$ | 500 | 171, 189 | 0.36 |
| 0.0064 | $5 \times 10^4$ | 0 | 0,0, 0,0 | 0 |
| 0.032 | 0 | 500 | 153 | 0.31 |
| 0.032 | $5 \times 10^4$ | 500 | 207 | 0.41 |

the efficiency of plating of Am-resistant cells is not significantly affected by the presence of $5 \times 10^4$ Am-sensitive cells on the plate; the presence of Am-resistant cells in the parental population should therefore have been detected. It can thus be concluded that the proportion of Am-resistant cells in the parental *St1* population remained always below a value of $10^{-4}$.

Cell populations with an increased resistance to aminopterin could be obtained from the parental *St1* line by exposing populations of 2–4 million *St1* cells to the drug and by varying either the length of exposure time or the concentration of the drug. Cell clones resistant to a concentration up to 0.512 μg/ml were obtained in successive selection steps. A selection for resistance to higher levels of the drug has not been attempted.

The karyotype distributions have been studied for three cell populations with different degrees of resistance to aminopterin. They are given in Figure 4. All three Am lines were started from parallel *St1* cultures two to three months after the isolation of the *St1* clone. The two main karyotypes found in the parental population at this time were a karyotype of 78 (12 large) and a karyotype of 79 (11 large) chromosomes (Figure 1 and VOGT 1958).

Line *Am 16–6* was exposed for 1–3 days to high doses of aminopterin, varying from 0.5 to 10 μg/ml. The exposures to the drug were spaced at 2–3 week intervals, the average time required until the survivors had grown out to form a confluent sheet. After four months, the culture was continuously grown in the presence of 0.008 μg Am/ml. The chromosome numbers and the aminopterin resistance of the line were determined five months after the selection had been started. As shown in Table 1, 56 percent of the colony formers of the line *Am 16–6*, as compared to 16 percent of the parental line, survived an aminopterin concentration of 0.0054 μg/ml. A small proportion of the cells were in addition able to form colonies in the presence of 0.0064 and 0.0080 μg Am/ml. The chromosome counts revealed two new classes of karyotypes (Figure 4) not encountered among the karyotypes of the parental *St1* population (Figure 1). It may be of interest to mention that the karyotype of 75 chromosomes carried a translocation in one of the largest chromosomes. In certain mitotic figures, this chromosome had the

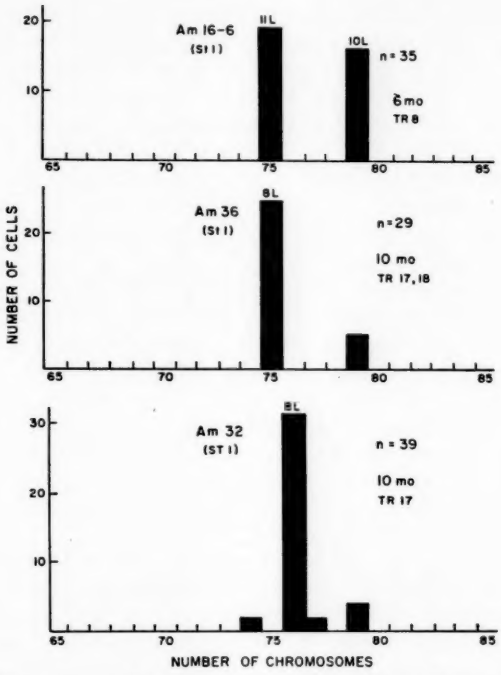


FIGURE 4.—Chromosome numbers in three populations with an increased resistance to the drug aminopterin.

appearance of a dicentric chromosome similar to that described by CHU and GILES (1958) for the clone S3-RA1.

Line *Am 36* was—within a three month period—exposed twice for one and three days, respectively, to 2.5 $\mu$g Am/ml. Subsequently the line was grown for three months in the continuous presence of aminopterin in concentrations increasing from 0.004, 0.008, 0.012, 0.016 to 0.032 $\mu$g/ml. During the seventh month, the aminopterin was omitted from the medium and the culture was allowed to grow for approximately 30 cell generations without drug. The intercalation of a growth period without drug was thought to eliminate any aminopterin-dependent cells from the population, should such cells have been present. The aminopterin resistance and the karyotype distribution of the population were determined after one and two passages in the presence of a concentration of 0.032 $\mu$g Am/ml immediately following the drug-free growth period. Table 2 shows that the survival of the population as colony formers was practically unaffected by a concentration of 0.032 $\mu$g Am/ml, the highest drug concentration at which the cells had been grown previously. The survival of the colony formers was reduced to 18 percent at a drug concentration of 0.128 $\mu$g/ml. Two karyo-

types were found in a sample of 29 cells (Figure 4). The most frequent karyotype with 75 (eight large) chromosomes was again a karyotype that had not been observed in the parental *St1* population and which differed also from the karyotype present in the *Am 16–6* population.

The selection procedure used to obtain line *Am 32* differed from that described for line *Am 36* only in two respects. The drug was not omitted during the seventh month of selection and the highest concentration of the drug used during the last month before the culture was analyzed was only 0.016 μg/ml. As shown in Table 1, the highest cell survival for line *Am 32* was obtained at a concentration of 0.016 μg Am/ml. It decreased to one third at 0.064 μg Am/ml. In contrast, only one percent of the plated cells were capable of giving rise to colonies in the absence of the drug, which indicates that the majority of the population was aminopterin dependent. The most frequent karyotype found in this cell line (Figure 4) was a karyotype with 76 (eight large) chromosomes. It differed from the karyotypes of the parental *St1* population and of the two other *Am*-lines.

The results show therefore that cultivation of the parental *St1* population in the presence of various concentrations of aminopterin led in three cases to populations with an increased resistance to aminopterin. The karyotype of the three selected populations differed from one another and from those of the parental *St1* population.

*Karyotypes of clones of different morphology:* Under hypothesis 2, discussed in the introduction, cells of different phenotype should have different karyotypes. To test the hypothesis, clones of different cell or colonial morphology were isolated from the two parental populations and their karyotypes determined within the first two months of cultivation. Figure 5 shows the chromosome counts obtained for five morphologically different clones isolated from a single-cell plating of the parental *St1* line at its 130th passage in standard medium.

Clone *St1–3I* had a phenotype intermediate between the polygonal and fusiform type and corresponded to the most frequent phenotype of the parental *St1* population at this late passage. Its main karyotype of 76 chromosomes (and 12 large chromosomes) is the same karyotype that was most frequently present at late passages of the parental *St1* line (Figure 1).

Clone *St1–8P* was characterized by polygonal cells and a less compact colonial morphology than that from the original *St1* clone. Clones *St1–1F*, *St1–4F*, and *St1–5F* all had fusiform cells. They differed in their growth characteristics from one another: the cells of clone *St1–1F* grew mostly in suspension, whereas the cells of clone *St1–4F* adhered to the glass and had a tendency to migrate away from one another, which in turn differentiated them from the more tightly packed growth of clone *St1–5F*. The sensitivity to poliovirus, as measured by the plaque-forming titer obtained for a standard virus suspension, was the same as that of the original *St1* line for clones *St1–3I*, *St1–5F*, and *St1–8P*. It was reduced to one third for clones *St1–1F* and *St1–4F*. As seen in Figure 5, each of the five morphologically different clones obtained from the parental *St1* line had a different karyotype.

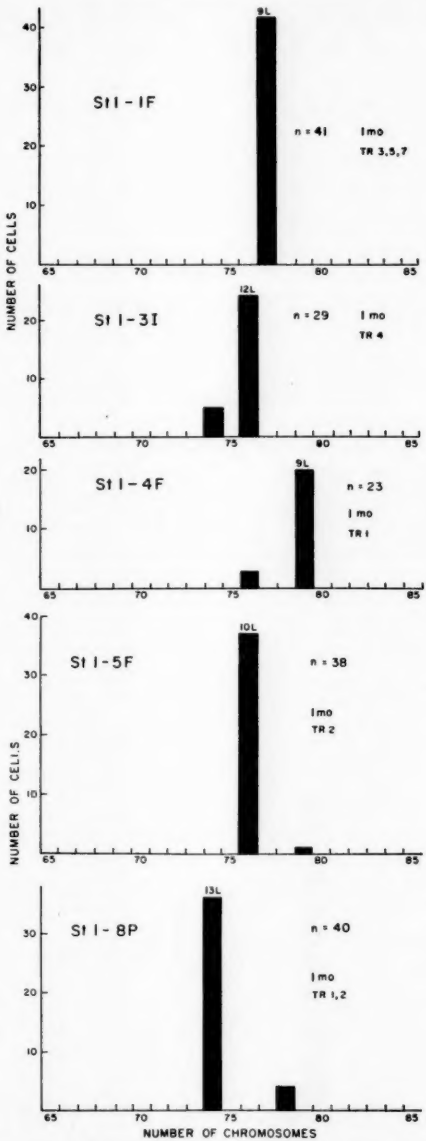


FIGURE 5.—Chromosome numbers in five different morphological clones selected from the parental *St1* line after 15 months of growth in standard medium.

Figure 6 shows the same for the karyotypes of two morphologically different clones isolated from the *F8(Ho)* line after two months of growth in standard medium. Clone *F8–4I* had the same intermediate phenotype as the prevalent type in the *F8(Ho)* population. Its karyotype was the same as that most frequently found in the *F8(Ho)* population (tr 148, Figure 2). Clone *F8–3F* had fusiform cells. Its colonial morphology was more compact than that of the original *F8* clone. The karyotype of clone *F8–3F* contained 69 chromosomes and differed thus from that of clone *F8–4I*.

## DISCUSSION

As discussed in the introduction, the hypothesis that the karyotype determines the phenotype in aneuploid cells would be supported by two findings: 1) that clones selected for different phenotypes have also different karyotypes, and 2) that cell lines selected and maintained under strong selection pressure maintain a relatively stable karyotype.

In the present study of two lines of HeLa cells, both findings were obtained in all cases. The first finding is not in disagreement with HAUSCHKA's (1958) report that in only three out of seven amethopterin resistant cell lines could a karyotype change be observed, since in the remaining four cases the changes could have involved a shift in the proportion of indistinguishable chromosome classes.

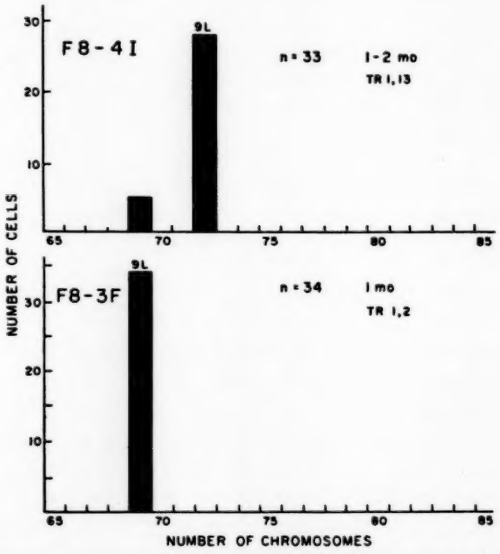


FIGURE 6.—Chromosome numbers of two morphologically different clones isolated from the *F8* (Ho) line at its 160th passage. (After eight months of growth in horse serum medium, the line had been grown for the last two months in standard medium.)

The second finding is especially significant, since it shows that the selective value of a cell depends on its karyotype. This is best explained if the karyotype directly determines the phenotype.

Furthermore it was found that whenever clones with the most frequent parental phenotype were isolated, their karyotype corresponded to that prevalent in the parental population (clones *St1–3I*, *F8–4I*, and *C1 7–3*). This finding argues against the possibility that the karyotype differences between morphologically different clones were artifacts, resulting either from the exceptional conditions or from the manipulations involved in the isolation of the clones.

The results described lend strong support to the view that the abundant variation observed in aneuploid cell populations, as for instance in many tumors, is mainly a consequence of irregular chromosome segregation and of the resulting shift in gene balance.

## SUMMARY

A comparative study of the karyotypes of two cloned lines of strain HeLa, S3, and their variant sublines has been made. A given karyotype was classified by the total number of chromosomes and by the number of large chromosomes, corresponding to the three largest chromosome pairs of the human idiogram. All variant sublines—two sublines adapted to growth in horse or calf serum as sole source of serum and three sublines showing an increased resistance to the drug aminopterin—were found to have karyotypes which had not been observed in the parental lines. Similarly, clones selected for their different morphology were found to differ in their karyotypes from the parental type and from one another. Two of the selected sublines maintained a stable karyotype while grown under strong selection pressure. All these findings support the hypothesis that the phenotypic variability of aneuploid cell populations is mainly caused by changes in the chromosomal constitutions of the cells.

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